PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
TCS-420.1PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/ 22145	20/10/1998	20/10/1997
Applicant		
AVANT IMMUNOTHERAPEUTICS, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 3

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 June 1999 (15.06.99)	
International application No. PCT/US98/22145	**Applicant's or agent's file reference** TCS-420.1PCT
International filing date (day/month/year) 20 October 1998 (20.10.98)	**Priority date** (day/month/year) 20 October 1997 (20.10.97)
Applicant RITTERSHAUS, Charles, W. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 May 1999 (18.05.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

C. Carrié

Telephone No.: (41-22) 338.83.38

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

YANKWICH, Leon R.
Yankwich & Associates
130 Bishop Allen Drive
Cambridge, Massachusetts 02139
ETATS-UNIS D'AMERIQUE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 14.01.00
Applicant's or agent's file reference TCS-420.1PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/22145	20/10/1998	20/10/1997

Applicant
AVANT IMMUNOTHERAPEUTICS, INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Danti, B Tel.+49 89 2399-8161

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PATENT COOPERATION TREATY

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REC'D 18 JAN 2000

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
TCS-420.1PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/22145	20/10/1998	20/10/1997
International Patent Classification (IPC) or national classification and IPC A61K39/00		
Applicant AVANT IMMUNOTHERAPEUTICS, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/05/1999	14.01.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Renggli, J Telephone No. +49 89 2399 7461

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US98/22145

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-40 as originally filed

Claims, No.:

1-37 as originally filed

Drawings, sheets:

1/17-17/17 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,5,8-14,23,24,26-37
	No:	Claims	1-3,6-7,15-22,25
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-37
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	15-37 (?)

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

ITEM V:

1. Reference is made to the following documents:

D1 Hesler et al., J. Biol. Chem., 1987, 262(5), 2275-2282
D2 Smith, A. M., Med. Sci. Res., 1993, 21, 911-912
D3 WO 96/39168
D4 WO 96/34888
D5 Hesler et al., J. Biol. Chem., 1988, 263 (11), 5020-5023
D6 WO 96/32932
D7 WO 96/15241

2. Industrial applicability (Art. 33(4) PCT):

The subject-matter of claims 1-14 is susceptible of industrial application.

For the assessment of the present claims 15-37 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

3. Preliminary remarks

The feature "non-endogenous" is not a feature which can render the vaccines of claims 1-14 novel. The said feature becomes relevant only upon the use of the composition or in other words, when the vaccine is injected into the mammalian subject (cf. Guidelines III- 4.8 PCT). However, the composition per se is not different and any document disclosing a vaccine containing any CETP (human, rabbit,...) is therefore prejudicial to the novelty of the claims containing the feature "non-endogenous".

4. Novelty (Art. 33(2) PCT):

D1 aims at characterizing the human plasma cholesteryl ester transfer protein (CETP). An immunological approach was undertaken to confirm that the 74 kD protein isolated was CETP. Rabbits were immunized with gel isolated human CETP in combination with RIBI adjuvant (cf. D1, page 2278, 1st column). The antibodies specific for the 74 kD protein were capable of inhibiting the CETP activity of partially purified plasma preparations (cf. D1, fig. 5, page 2278 and page 2282, supplementary material).
D1 is therefore prejudicial to the novelty of claims 15 and 25 of the present application (Art. 33(2) PCT). Claims 1-7 are considered novel over D1 which is not directed to the use of CETP as vaccine.

D2 discloses the preparation in rabbits of anti-peptide antibodies specific for the human CETP (residues 131-142). Rabbits were immunized with KLH-peptide conjugates in CFA/IFA and IgG were purified on an ProtA column. The antibodies generated in rabbits were capable of inhibiting CETP activity and recognized in Western Blot analysis a 72 kD protein (cf. D2, page 912, results and discussion).
D2 anticipates the subject-matter of claims 15 and 25 of the present application (Art. 33(2) PCT). Claims 1-7 are considered novel over D2 which is directed to the use of CETP for diagnostic purposes (cf. D2, abstract and page 912 , last paragraph) and does not mention its use in a vaccine composition.

D3 describes a method for inducing an immune response against CETP for increasing the HDL level in the serum (cf. D3, page 2, lines 18-22). This is achieved by the immunization of mammals with CETP or fragments of CETP, optionally with an adjuvant (cf. page 2, line 26-page 3, line 2). The sequences injected in D3 are homologous to the CETP sequences or sufficiently homologous to inhibit the activity of CETP (cf. D3, page 5, lines 11-21). The immunization of rabbits with human peptide-OVA conjugates in CFA/IFA is disclosed in example 1 (page 7); alternatively, rabbits were immunized with the human/rabbit homologous peptide 3 in combination with tetanus toxoid. The animals immunized with the latter tetanus conjugate had (i) a lower CETP activity, (ii) a higher HDL-cholesterol level, (iii) a stable LDL-cholesterol level and (iv) a higher HDL/LDL ratio (cf. D3, pages 14-15).
The subject-matter of claims 1-3, 6, 7, 15-21 and 25 of the present application is therefore anticipated by D3 (Art. 33(2) PCT).

D4 is directed to a vaccine for inducing an immune response against endogenous CETP in humans and animals (cf. page 1). D4 discloses a peptide vaccine comprising a universal T helper epitope and an endogenous CETP B epitope (cf. page 6). The vaccine can be injected in combination with a pharmaceutically acceptable adjuvant (cf. page 7, lines 28-29 and page 22). The peptide vaccine of D4 enables the reduction of the ratio non-HDL/HDL and of the atherosclerotic lesions (cf. figs. 6 and 13, examples 9 and 10). The use of endogenous, non-endogenous cross-reactive and xenogeneic CETP (rabbit and human) is described in D4 (page 12, line 27-page 13, line 18 / example 2, page 28 / example 6, page 32 / example 7, pages 36-37). D4 is therefore prejudicial to the novelty of claims 1-3, 6, 7, 15-22 and 25 (Art. 33(2) PCT).

D5 discloses a method for producing antibodies against human CETP in mice (cf. abstract and materials and methods, page 5020). This document is therefore prejudicial to the novelty of claims 15 and 25 of the present application (Art. 33(2) PCT).

5. Inventive step:

D3 and D4 are considered as the closest prior art documents.

The subject-matter of claims 4 and 5 is new over D3/D4 which do not disclose a humanized (modified) rabbit CETP or the specific sequence ID No.5.
The problem solved by claims 4 and 5 appears to be the provision of alternatives to the existing constructions of D3/D4. The solution consists in the use of humanized rabbit CETP like seq ID No. 5. The solution is not linked to any surprising technical effects. This solution is trivial for the skilled person wishing to develop an alternative to D3/D4. The elimination of potentially undesirable immunoreactive regions is commonplace in the field (e.g. humanized monoclonal antibodies) and the subject-matter of claims 4 and 5 is not considered inventive within the meaning of Article 33(3) PCT. It follows that claims 23 and 24, directed to known therapeutical methods (cf. D3 and D4), are not inventive either.

The subject-matter of claims 8-14 and 26-37 is novel over D1-D5. None of the cited documents discloses the use of plasmid based vaccines encoding a CETP protein.

The subject-matter of the said claims provides an alternative to the vaccines and

related methods of treatments of D3 and D4. The solution consist in the use of standard plasmid based vaccines under the control of a promoter.
The use of plasmid based vaccines for immunizing is known (cf. D6 and D7) and the solution is therefore trivial for the skilled person wishing to provide an alternative to D3 and D4. The alternatives are not linked to any surprising technical effects and therefore, the subject-matter of claims 8-14 (cf. D4, page 21) and 26-37 (cf. D3 and D4) is not inventive within the meaning of Article 33(3) PCT.

6. Priority and P documents (Art. 8 PCT):

The present opinion is given assuming that the claimed priority is valid. The document Thomas L. J. et al., Use of xenogeneic cholesteryl ester transfer protein (CETP) in a plasmid-based vaccine to produce anti-cetp autoantibodies for the prevention/treatment of atherosclerosis' FASEB JOURNAL, vol. 12, no. 4, 1998, page a310,1805 is therefore not relevant for the examination of novelty and inventive step at present. However, the attention of the applicant is drawn to the fact that this document may be relevant in the examination of novelty and inventive step for those parts of the application, if any, which do not have a valid claim to priority.

ITEM VI:

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 97/41227	06.11.97	01.05.97	01.05.96/21.02.97

The above document, which has an earlier priority date than the present application may become relevant (i) in the regional phase of the application and (ii) may be relevant in the examination of novelty and inventive step for those parts of the application, if any, which do not have a valid claim to priority.

ITEM VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 are not mentioned in the description, nor

are these documents identified therein.

2. The vague statements in the description on page 22, lines 8-11 "...the spirit and the scope of the invention..." and on page 4, line 25 and page 22, line 11 "...incorporated by reference..." implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, C-III, 4.3a and C-II, 4.17).

ITEM VIII:

1. Claims 1-37 are not supported by the description as required by Article 6 PCT, as their scope is broader than justified by the description. The present application is directed to the use of **whole** CETP molecules of another mammal (cf. description of the present application, page 5, lines 7-14 and page 14, line 26-page 15, line 4) to elicit antibodies against endogenous CETP. This limitation is not present in the claims and the said claims contravene therefore to the requirements of Article 6 PCT, Guidelines C-III 4.3 PCT.

2. Claims 2-4, 9, 11, 15-21, 23 and 34 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem:

   -mammalianized CETP
   -humanized CETP

   The technical features necessary for achieving this result should be present in the said claims.

3. As explained above (cf. section V, item 3), some of the features in the vaccine claims 1-14 relate to a method of using the vaccine rather than clearly defining the vaccines in terms of their technical features. The intended limitations are therefore not clear from these claims, contrary to the requirements of Article 6 PCT. Moreover, the features of claims 2, 6, 9, 10, 11 and 13 relate exclusively to the

particular use of the vaccines claimed; these features are therefore irrelevant with respect to the products which have to be defined in terms of their technical features (cf. Guidelines C-III, 2.1, 4.1, 4.8 PCT)

4. The term "essentially" used in claims 1 and 3 is vague and unclear and leaves the reader in doubt as to the content of the compositions, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

HB

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A61K 39/00, 39/39, 48/00, C07K 14/47	A1	WO 99/20302
		(43) International Publication Date: 29 April 1999 (29.04.99)

(21) International Application Number: PCT/US98/22145

(22) International Filing Date: 20 October 1998 (20.10.98)

(30) Priority Data:
08/954,643 20 October 1997 (20.10.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 08/954,643 (CIP)
Filed on 20 October 1997 (20.10.97)

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(75) Inventors/Applicants *(for US only)*: RITTERSHAUS, Charles, W. [US/US]; 65 Garden Street, Malden, MA 02148 (US). THOMAS, Lawrence, J. [US/US]; 1 Fox Ridge Road, Easton, MA 02375 (US).

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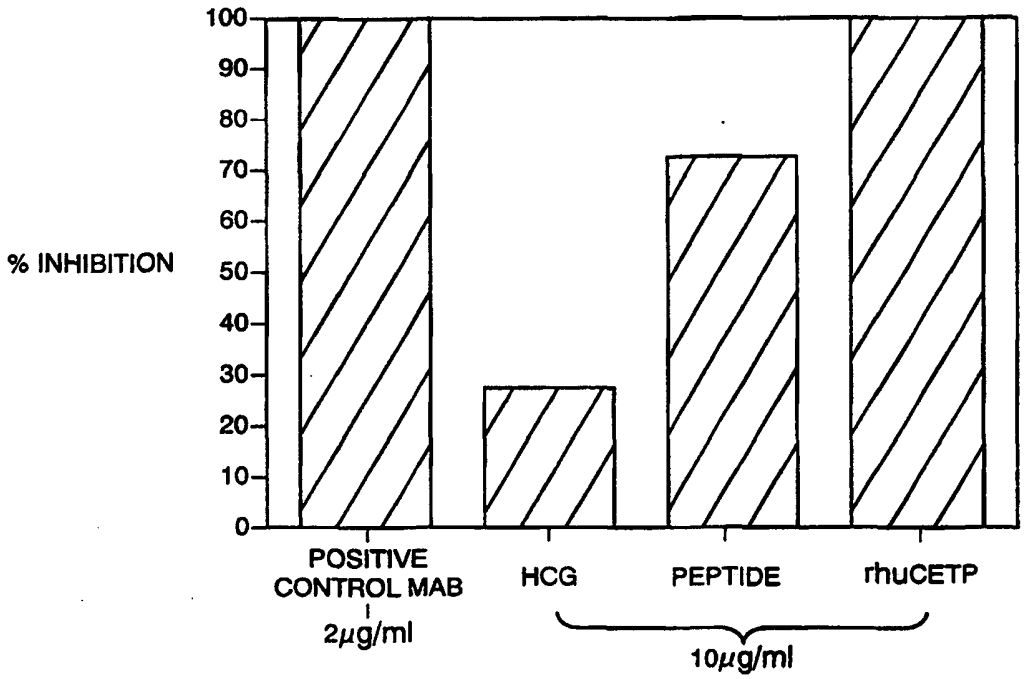
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: XENOGENEIC CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) FOR MODULATION OF CETP ACTIVITY



(57) Abstract

Methods for modulating cholesteryl ester transfer protein (CETP) activity and the plasma levels of lipoproteins involved in heart disease involve administration of a non–endogenous CETP or a plasmid–based vaccine for expression of such non–endogenous CETP to elicit production in a mammal of antibodies that recognize (bind to) the mammal's native (endogenous) CETP.

FOR THE PURPOSES OF INFORMATION ONLY

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XENOGENEIC CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) FOR MODULATION OF CETP ACTIVITY

Cross-Reference to Related Application

This application is a continuation-in-part application of United States Patent Application Serial No. 08/954,643, filed 20 October 1997.

Field of the Invention

This invention relates to the field of cardiovascular disease, particularly atherosclerosis. More particularly, the present invention provides compositions and methods for the control, treatment, or reduction of the risk of atherogenic activity in the circulatory system of mammals, particularly humans.

Background of the Invention

Cholesterol circulates through the body predominantly as a component of lipoprotein particles (lipoproteins), which are composed of a protein portion, called apolipoproteins (Apo) and various lipids, including phospholipids, triglycerides, cholesterol and cholesteryl esters. There are ten major classes of apolipoproteins: Apo A-I, Apo A-II, Apo-IV, Apo B-48, Apo B-100, Apo C-I, Apo C-II, Apo C-III, Apo D, and Apo E. Lipoproteins are classified by density and composition. For example, high density lipoproteins (HDL), one function of which is to mediate transport of cholesterol from peripheral tissues to the liver, have a density usually in the range of approximately 1.063-1.21 g/ml. HDL contain various amounts of Apo A-I, Apo A-II, Apo C-I, Apo C-II, Apo C-III, Apo D, Apo E, as well as various amounts of lipids, such as cholesterol, cholesteryl esters, phospholipids, and triglycerides (TG).

In contrast to HDL, low density lipoproteins (LDL), which generally have a density of approximately 1.019-1.063 g/ml, contain Apo B-100 in association with various lipids. In particular, the amounts of the lipids, cholesterol, and cholesteryl esters are considerably higher in LDL than in HDL when measured as a percentage of dry mass. LDL are particularly important in delivering cholesterol to peripheral tissues.

Very low density lipoproteins (VLDL) have a density of approximately 0.95-1.006 g/ml and also differ in composition from other classes of lipoproteins both in their protein and lipid content. VLDL generally have a much higher amount of triglycerides than do HDL or

LDL and are particularly important in delivering endogenously synthesized triglycerides from liver to adipose and other tissues. The features and functions of various lipoproteins have been reviewed (see, for example, Mathews and van Holde, Biochemistry, pp. 574-576 and 626-630 (The Benjamin/Cummings Publishing Co., Redwood City, California, 1990); Havel et al., "Introduction: Structure and metabolism of plasma lipoproteins", in The Metabolic Basis of Inherited Disease, 6th ed., pp. 1129-1138 (Scriver et al., eds.) (McGraw-Hill, Inc., New York, 1989); Zannis et al., "Genetic mutations affecting human lipoproteins, their receptors, and their enzymes", in Advances in Human Genetics, Vol. 21, pp. 145-319 (Plenum Press, New York, 1993)).

Decreased susceptibility to cardiovascular disease, such as atherosclerosis, is generally correlated with increased absolute levels of circulating HDL, with lowered levels of LDL or VLDL, and also with increased levels of HDL relative to circulating levels of VLDL and LDL (see, e.g., Gordon et al., *N. Engl. J. Med.*, 321: 1311-1316 (1989); Castelli et al., *J. Am. Med. Assoc.*, 256: 2835-2838 (1986); Miller, et al., *Am. Heart J.*, 113: 589-597 (1987); Tall, *J. Clin. Invest.*, 89: 379-384 (1990); Tall, *J. Internal Med.*, 237: 5-12 (1995)).

Cholesteryl ester transport protein (CETP) mediates the transfer of cholesteryl esters from HDL to TG-rich lipoproteins such as VLDL and LDL, and also the reciprocal exchange of TG from VLDL to HDL (Tall, *ibid.*; Tall, *J. Lipid Res.*, 34: 1255-1274 (1993); Hesler et al., *J. Biol. Chem.*, 262: 2275-2282 (1987); Quig et al., *Ann. Rev. Nutr.*, 10: 169-193 (1990)). CETP may play a role in modulating the levels of cholesteryl esters and triglyceride associated with various classes of lipoproteins. A high CETP cholesteryl ester transfer activity has been correlated with increased levels of LDL-associated cholesterol and VLDL-associated cholesterol, which in turn are correlated with increased risk of cardiovascular disease (see, e.g., Tato et al., *Arterioscler. Thromb. Vascular Biol.*, 15: 112-120 (1995)).

CETP isolated from human plasma is a hydrophobic glycoprotein having 476 amino acids and a molecular weight of approximately 66,000 to 74,000 daltons on sodium dodecyl sulfate (SDS)-polyacrylamide gels (Albers et al., *Arteriosclerosis*, 4: 49-58 (1984); Hesler et al., *J. Biol. Chem.*, 262: 2275-2282 (1987); Jarnagin et al., *Proc. Natl. Acad. Sci. USA*, 84: 1854-1857 (1987)). A cDNA encoding human CETP has been cloned and sequenced (see, Drayna et al., *Nature*, 327: 632-634 (1987)). CETP has been shown to bind choesteryl esters, trygicerides, phospholipids (Barter et al., *J. Lipid Res.*, 21:238-249 (1980)), and lipoproteins (see, e.g., Swenson et al., *J. Biol. Chem.*, 264: 14318-14326 (1989)). More recently, the

region of CETP defined by the carboxyl terminal 26 amino acids, and in particular amino acids 470 to 475, has been shown to be especially important for neutral lipid binding involved in neutral lipid transfer (Hesler et al., *J. Biol. Chem.*, 263: 5020-5023 (1988)).

Hereinafter, LDL-C will be used to refer to total cholesterol, including cholesteryl esters and/or unesterified cholesterol, associated with low density lipoprotein. VLDL-C will be used to refer to total cholesterol, including cholesteryl esters and/or unesterified cholesterol, associated with very low density lipoprotein. HDL-C will be used to refer to total cholesterol, including cholesteryl esters and/or unesterified cholesterol, associated with high density lipoprotein.

A number of *in vivo* studies utilizing animal models or humans have indicated that CETP activity can affect the level of circulating cholesterol-containing HDL. Increased CETP cholesteryl ester transfer activity can produce a decrease in HDL-C levels relative to LDL-C and/or VLDL-C levels which in turn is correlated with an increased susceptibility to atherosclerosis. Injection of partially purified human CETP into rats (which normally lack CETP activity), resulted in a shift of cholesteryl ester from HDL to VLDL, consistent with CETP-promoted transfer of cholesteryl ester from HDL to VLDL (Ha et al., *Biochim. Biophys. Acta*, 833: 203-211 (1985); Ha et al., *Comp. Biochem. Physiol.*, 83B: 463-466 (1986); Gavish et al., *J. Lipid Res.*, 28: 257-267 (1987)). Transgenic mice expressing human CETP were reported to exhibit a significant decrease in the level of cholesterol associated with HDL (see, e.g., Hayek et al., *J. Clin. Invest.*, 90: 505-510 (1992); Breslow et al., *Proc. Natl. Acad. Sci. USA*, 90: 8314-8318 (1993)). Furthermore, whereas wild-type mice are normally highly resistant to atherosclerosis (Breslow et al., *ibid.*), transgenic mice expressing a simian CETP were reported to have an altered distribution of cholesterol associated with lipoproteins, namely, elevated levels of LDL-C and VLDL-C and decreased levels of HDL-C (Marotti et al., *Nature*, 364: 73-75 (1993)). Transgenic mice expressing simian CETP also were more susceptible to dietary-induced severe atherosclerosis compared to non-expressing control mice and developed atherosclerotic lesions in their aortas significantly larger in area than those found in the control animals and having a large, focal appearance more typical of those found in atherosclerotic lesions in humans (Marotti et al., *ibid.*). Intravenous infusion of anti-human CETP monoclonal antibodies (Mab) into hamsters and rabbits inhibited CETP activity *in vivo* and resulted in significantly increased levels of HDL-C, decreased levels of HDL-triglyceride, and increased HDL size; again implicating a critical role for CETP in the

distribution of cholesterol in circulating lipoproteins (Gaynor et al., *Atherosclerosis*, 110: 101-109 (1994) (hamsters); Whitlock et al., *J. Clin. Invest.*, 84: 129-137 (1989) (rabbits)).

CETP deficiency has also been studied in humans. For example, in certain familial studies in Japan, siblings that were homozygous for non-functional alleles of the CETP gene had no detectable CETP activity. Virtually no atherosclerotic plaques were exhibited by these individuals, who also showed a trend toward longevity in their families (see, e.g., Brown et al., *Nature*, 342: 448-451 (1989); Inazu et al., *N. Engl. J. Med.*, 323: 1234-1238 (1990); Bisgaier et al., *J. Lipid Res.*, 32: 21-23 (1991)). Such homozygous CETP-deficient individuals also were shown to have an anti-atherogenic lipoprotein profile as evidenced by elevated levels of circulating HDL rich in cholesteryl ester, as well as overall elevated levels of HDL, and exceptionally large HDL, i.e., up to four to six times the size of normal HDL (Brown et al., *supra*, p. 451). The frequency of this mutation in Japan is relatively high, and may account for an elevated level of HDL in a significant fraction of the Japanese population.

The above studies indicate that CETP plays a major role in transferring cholesteryl ester from HDL to VLDL and LDL, and thereby in altering the relative profile of circulating lipoproteins to one which is associated with an increased risk of cardiovascular disease (e.g., decreased levels of HDL-C and increased levels of VLDL-C and LDL-C). Taken together, the current evidence suggests that increased levels of CETP activity may be predictive of increased risk of cardiovascular disease. Modulation or inhibition of endogenous CETP activity is thus an attractive therapeutic method for modulating the relative levels of lipoproteins to reduce or prevent the progression of, or to induce regression of, cardiovascular diseases, such as atherosclerosis.

In our previous work, detailed, e.g., in commonly assigned, copending patent application PCT/US96/06147 (WO 96/34888) and commonly assigned copending patent application PCT/US97/07294 (WO 97/41227), both incorporated herein by reference, we detailed an approach for modulating the CETP activity in an individual via vaccination with a peptide composition or with a plasmid-based vaccine that would lead to the production of antibodies recognizing and neutralizing endogenous CETP. We demonstrated that administration of immunogenic peptides either by direct inoculation or by *in situ* production following injection of a functional plasmid-based vaccine resulted in production of antibodies reactive with the inoculated individual's own (endogenous) CETP. Thus the vaccine peptides and the plasmid-based vaccines break tolerance in the vaccinated individuals and to promote

production of antibodies recognizing a self protein. Furthermore, administration of these vaccines to test animals resulted in a decline in the relative levels of total cholesterol and HDL-C and resulted in a decrease in the development of atherosclerotic lesions. The elicited endogenous anti-CETP antibodies thus promote a physiological condition correlated with decreased risk of cardiovascular disease, and they appear to have a direct effect on preventing or decreasing the formation of atherosclerotic plaques.

We have now discovered another approach to eliciting the production of anti-CETP antibodies in a mammal. We have now determined that whole CETP molecules of another mammal, that is, non-endogenous CETP, can be used to elicit antibodies in a mammal that will be reactive with its own, endogenous CETP and serve to modulate the activity of CETP and to provide lowered circulating CETP activity, lowered total cholesterol, lowered circulating LDL levels, elevated ratios of HDL-C to LDL-C. The use of non-endogenous CETP to promote production of anti-endogenous CETP antibodies also leads to a reduction in development of atherosclerotic lesions in comparison to unvaccinated controls.

Summary of the Invention

Accordingly, the present invention provides compositions and methods useful for the modulation or inhibition of cholesteryl ester transfer protein (CETP) activity. In particular, the use of non-endogenous CETPs, including xenogeneic CETPs, is described as a means, when administered to a mammal, to raise an antibody response against the mammal's own endogenous CETP and thereby to promote a prophylactic or therapeutic effect against cardiovascular disease, such as atherosclerosis. Such non-endogenous CETP can be CETP of another mammalian species (xenogeneic CETP), such as rabbit CETP, mouse CETP or simian CETP for administration to a human; the non-endogenous CETP can be a non-endogenous allelic variation or polymorph of a mammalian CETP administered to the same species of mammal (e.g., a human CETP polymorph administered to another human); or the non-endogenous CETP can be a CETP from one species modified to have an amino acid sequence more similar to the native CETP of another species (e.g., a "humanized" rabbit CETP for administration to a human).

Vaccine compositions and plasmid-based vaccines are described which, when suitably administered to a mammal result in the production of antibodies reactive with the mammal's endogenous CETP and the other benefits described herein.

The invention provides methods for eliciting antibodies in a mammal that will be reactive with its own, endogenous CETP, for modulating the activity of CETP in a mammal, and for providing in a mammal lowered circulating CETP activity, lowered total cholesterol, lowered circulating LDL levels, and/or elevated ratios of HDL-C to LDL-C. The invention provides a method for reducing or preventing in a mammal the development of atherosclerotic lesions.

Brief Description of the Drawings

Figure 1A-C. An alignment of the amino acid sequences of mature rabbit CETP (SEQ ID NO: 3) with mature human CETP (SEQ ID NO: 1). The rabbit CETP is shown over the aligned human CETP sequence. The rabbit sequence includes 20 more amino acid residues than the human sequence, and the human sequence shows a 1-amino acid and a 19-amino acid gap (indicated with dashes, ---, in the human sequence) in order to show the residue matches (indicated with a vertical line, |) most clearly.

Figure 2. Shows the end point titers of antibodies from rabbit plasma recognizing the C-terminal peptide of rabbit CETP from rabbits vaccinated with human chorionic gonadotropin ("HCG Vaccine"), a synthetic vaccine peptide having segments of tetanus toxoid and the C-terminal sequence of human CETP ("Peptide Vaccine", see SEQ ID NO: 7), and full-length recombinant human CETP ("rhuCETP"). The figure shows maximum anti-CETP antibody titers achieved for each rabbit in an ELISA detecting plasma antibodies specific for rabbit CETP C-terminal peptide (amino acids 477-496).

Figure 3. Shows inhibition of CETP activity in a commercial fluorescence-based assay (Roar Biomedical, Yonkers, New York) by protein A-isolated antibodies from the plasma of vaccinated rabbits from Groups I-III (see Examples, *infra*). The graph shows percent inhibition achieved in each of the vaccinated rabbits.

Figure 4. Shows the CETP activity in the vaccinated rabbits from week 1 to week 32.

Figure 5. Shows the percentage change in total cholesterol levels in the vaccinated rabbits from week 1 to week 12.

Figure 6. Shows the HDL-associated cholesterol levels in the vaccinated rabbits from week 1 to week 32.

Figure 7. Shows the percentage change in LDL-associated cholesterol levels in the vaccinated rabbits from week 1 to week 12.

Figure 8. Shows the plasma lipoprotein levels for a rabbit vaccinated with non-endogenous CETP(recombinant human CETP, or rhuCETP). "V" indicates the periodic vaccination boosts. HDL-associated cholesterol, total cholesterol level, and triglyceride level were assayed.

Figure 9. Shows a correlation between CETP activity and HDL as a percent of total lipoproteins and total CETP mass, in a rabbit vaccinated with non-endogenous CETP (rhuCETP)

Figure 10. Shows the levels of cholesterol deposits in the irises of 48 rabbits vaccinated with human chorionic gonadotropin ("HCG", rabbits #1-#12), a synthetic vaccine peptide having segments of tetanus toxoid and the C-terminal sequence of human CETP ("Peptide", see SEQ ID NO: 7, rabbits #13-#24), full-length recombinant human CETP ("rhuCETP", rabbits #25-#36), and a CETP-tetanus toxoid conjugate composition.("Conjugate", rabbits #37-#48). The irises of each rabbit have been scored based on the percentage of deposits observed from 0-5, 0 representing no deposits and 5 representing 100% deposits observed in the iris of the animal.

Figure 11. Shows percentage of the aorta covered by lesions observed in vaccinated rabbits fed an atherogenic diet. Values of individual animals are represented by open symbols.

Figure 12. Shows anti-CETP antibody titers for mice vaccinated with various plasmid-based vaccines.

Figure 13A-K. Shows antibody titers of 11 rabbits vaccinated with pSV40-HuCETP between weeks 1-32. The open diamond and open square symbols refer to the antibody titers of the rabbits on weeks 30 and 34 respectively.

Figure 14. Shows the effect of plasmid vaccination on the development of aortic lesions. The figure compares the mean percentages collected for control rabbits (pSV40 plasmid without the CETP coding sequence) and rabbits vaccinated with pSV40-huCETP.

Detailed Description of the Invention

As noted above, a decreased risk of atherosclerosis has been correlated with relatively low circulating levels of LDL and VLDL and relatively high levels of HDL. The levels of such circulating lipoproteins are directly influenced, at least in part, by the endogenous levels of CETP activity. In particular, high CETP activity promotes transfer of neutral lipids, such

as cholesteryl esters from HDL to VLDL and LDL. Accordingly, CETP is a relatively precise target in humans and other animals for altering the relative levels of LDL, VLDL and HDL in the circulatory system (see, e.g., Tato et al., *Arteriosclero. Thromb. Vascular Biol.*, 15: 112-120 (1995); Tall, *J. Internal Med.*, 237: 5-12 (1995)). This invention is directed to the control of endogenous CETP activity by providing non-endogenous CETP molecules to an individual, for promoting an immune response in such individuals against their endogenous CETP, thereby promoting a physiological condition (e.g., increased level of HDL or decreased level of LDL) correlated with a decreased risk of atherosclerosis. In addition, promoting an immune response against endogenous CETP using the vaccine compositions of this invention can provide, prevent, or inhibit the progression of lesions in tissue susceptible to atherosclerosis.

Compositions for Modulation of CETP Activity

As used herein, a CETP vaccine composition for use according to the invention contains as an essential ingredient a CETP or a portion thereof, that is non-endogenous with respect to the mammal to be vaccinated. For the purposes of this invention, "non-endogenous CETP" means a cholesteryl ester transfer protein that is not the native CETP produced by the mammal to be vaccinated. For example, with respect to a human subject, non-endogenous CETP will include CETP produced by another mammalian species, i.e., xenogeneic CETP, such as rabbit, mouse or simian CETP; or non-endogenous CETP with respect to a particular human subject can be an allelic variant or polymorphism of human CETP, such as CETP produced by another human individual.

The non-endogenous CETP can also be a xenogeneic CETP that has been modified in order to make the amino acid sequence of the modified CETP more similar to that of the endogenous CETP of the mammal to be vaccinated. The term used herein to describe such modified non-endogenous CETP is "mammalianized CETP". This term is used with reference to the mammal to be vaccinated, and it means a non-endogenous CETP that has been modified to have an amino acid content more similar to the native CETP of said mammal. An example of a mammalianized CETP, where the subject to be vaccinated is a human, would be a rabbit CETP modified (or "humanized") to have an amino acid sequence more similar to the native human sequence. As a further example, reference is made to Figures 1A, 1B and 1C, which show the respective amino acid sequences of rabbit CETP (SEQ ID NO: 3) and human CETP (SEQ ID NO: 1) in alignment.

Referring to Figures 1A-1C, it is seen that the structure of these two mammalian CETPs is similar, having the same amino acids at 80% of the positions of human CETP. Rabbit CETP (SEQ ID NO: 3) is 20 amino acids longer than human CETP (SEQ ID NO: 1), and the alignment of the two proteins in Figures 1A-1C shows two segments, denoted with dashes (----), where the proteins do not correspond structurally. With respect to a human subject to be administered a non-endogenous CETP in accordance with this invention, an example of a "mammalianized" non-endogenous CETP would be a rabbit CETP in which the 19-amino acid segment from amino acid Ala_{393} through Ala_{411} of rabbit CETP has been deleted, making the modified CETP (see SEQ ID NO: 5) 477 amino acids in length and thus more similar to the human CETP (SEQ ID NO: 1). Since the mammal of this example is a human, another term for such a modified CETP or mammalianized non-endogenous CETP would be a "humanized rabbit CETP".

Again referring to Figures 1A-1C, a further example of a humanized rabbit CETP would be a CETP as set forth in SEQ ID NO: 6. In Figure 1C it is noted that in the C-terminal portion of the human and rabbit CETPs there is only one difference in the respective amino acid sequences, i.e., Lys_{485} of the rabbit CETP corresponds to Glu_{465} in the human CETP.

In practicing the methods of the present invention, non-endogenous CETP is administered to a mammal in an amount effective to elicit an immune response. As is common in the field, more than one administration may be necessary or desirable to obtain a high enough concentration of anti-endogenous CETP antibodies in the mammal to affect endogenous CETP activity.

Immunogenicity of a vaccine peptide of this invention may be further enhanced by linking the immunogenic non-endogenous CETP to itself or to a related protein homologous to CETP. In this approach, a dimer could be formed, with the dimer providing a multi-chain protein that is even more immunogenic than the non-endogenous CETP alone. Examples of proteins related to CETP that might be used in this approach include, for example, phospholipid transfer protein and neutrophil bactericidal protein (see, Day et al., *J. Biol. Chem.*, 269: 9388-91 (1994); Gray et al., *J. Biol. Chem.*, 264: 9505-9509 (1989)).

Other immunogenic carrier molecules such as keyhole limpet hemocyanin (KLH) may also be used in combination with the non-endogenous CETP. For example, KLH contains multiple lysine residues in its amino acid sequence. Each of these lysines is a potential site at

which a CETP molecule as described herein could be linked (for example, using maleimide activated KLH, Catalog No. 77106, Pierce, Rockford, IL), to provide a multivalent non-endogenous CETP immunogen. Another example of an immunogenic carrier molecule is hsp70 from *Mycobacterium tuberculosis*, which has been shown to be an especially potent antigen containing multiple B and T cell epitopes (see, e.g., Suzue and Young, *J. Immunol.*, 156: 873 - 879 (1996)). The hsp70 protein can be linked by standard cross-linking agents to non-endogenous CETPs to enhance immunogenicity of the vaccine compositions.

Other peptides can be conjugated with the non-endogenous CETP molecules to provide a source of helper T cell epitopes and boost the immunogenicity of the vaccine compositions according to the invention. Such peptides include, for example, "universal" antigenic peptides, e.g., tetanus toxoid or diphtheria toxoid, especially the tetanus toxoid fragment: Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu (amino acids 2 to 15 of SEQ ID NO: 7).

Pharmaceutically acceptable adjuvants, such as alum, may be mixed with the non-endogenous CETPs described herein to produce vaccine compositions of this invention. Alum is the single adjuvant currently approved for use in administering vaccines to humans (see, Eldrige et al., in Immunobiology of Proteins and Peptides V: Vaccines: Mechanisms, Design, and Applications (Atassi, M.Z., ed.)(Plenum Press, New York, 1989), page 192). Alum in combination with a sodium phthalyl derivative of lipopolysaccharide can also be used (see, Talwar et al., *Proc. Natl. Acad. Sci. USA*, 91: 8532-8536 (1994)). Other conventional adjuvants may be used as they are approved for a particular use. For example, biodegradable microspheres comprised of poly (DL-lactide-co-glycolide) (Eldridge et al., *supra*, pp. 191-202); Freund's Complete Adjuvant (Sigma Chemical Co., St. Louis, Missouri), Freund's Incomplete Adjuvant (Sigma Chemical Co., St. Louis, Missouri), and the RIBI™ Adjuvant System (RAS; RIBI ImmunoChem Research, Inc., Hamilton, Montana); lipophilic N-palmitoyl-S-[2,3-bis(palmitoyloxy)-propyl)]-cysteine ("Pam_3-Cys-OH"); amphiphilic, water-soluble lipopeptides such as Pam_3-Cys-Ser-Lys_4 and Pam_3-Cys-Ser-Glu_4; glycopeptides such as N-acetyl-glucosaminyl-N-acetylmuramyl-alanyl-D-isoglutamine ("GMDP"), muramyl dipeptides, and alanyl-N-adamantyl-D-glutamine; and polyamide gel-based adjuvants which can easily be attached to CETP peptides during their *in vitro* chemical synthesis (see, Synthetic Vaccines (Nicholson, ed.) (Blackwell Scientific Publications, Cambridge, Massachusetts, 1994), pp. 236-238).

Where helper T cell epitope molecules or adjuvant species are to be physically linked or conjugated with the non-endogenous CETP, the CETP can be covalently linked directly or via a cross-linker molecule.

Suitable cross-linking molecules include amino acids, for example, using one or more glycine residues to form a "glycine bridge" between the CETP and the carrier or adjuvant molecule. Also contemplated are the formation of disulfide bonds between cysteine residues, or the use of cross-linking molecules such as glutaraldehyde (see, Korn et al., *J. Mol. Biol.*, 65: 525-529 (1972)) and EDC (Pierce, Rockford, IL) or other bifunctional cross-linker molecules. Bifunctional cross-linker molecules possess two distinct reactive sites; one of the reactive sites can be reacted with a functional group on the CETP and the other reactive site can be reacted with a functional group on the carrier or adjuvant molecule, uniting the two. General methods for cross-linking molecules are reviewed by Means and Feeney (*Bioconjugate Chem.*, *1*: 2-12 (1990)).

Homobifunctional cross-linker molecules have two reactive sites which are chemically the same. Examples of homobifunctional cross-linker molecules include glutaraldehyde; N,N'-bis(3-maleimido-propionyl)-2-hydroxy-1,3-propanediol (a sulfhydryl-specific homobifunctional cross-linker); certain N-succinimide esters, such as disuccinimidyl suberate and dithio-bis-(succinimidyl propionate) and their soluble bis-sulfonic acids and salts (e.g., as available from Pierce Chemicals, Rockford, Illinois; or Sigma Chemical Co., St. Louis, Missouri).

Preferably, the bifunctional cross-linker molecule is a heterobifunctional linker molecule, meaning that the linker molecule has at least two reactive sites that can be separately covalently attached to the non-endogenous CETP and the carrier or adjuvant molecule. Heterobifunctional cross-linker molecules that may be used include m-maleimidobenzoyl-N-hydroxysuccinimide ester; m-maleimido-benzoylsulfosuccinimide ester; γ-maleimidobutyric acid N-hydroxysuccinimide ester; and N-succinimidyl 3-(2-pyridyl-dithio)propionate.

The non-endogenous CETP for use according to this invention can be produced by any of the available methods known in the art to produce proteins of defined amino acid sequence. For example, automated peptide synthesis is available to those skilled in the art by using automated peptide synthesizers (e.g., Synergy™ Peptide Synthesizer by Applied Biosystems; AMS 422 by Abimed, Langenfeld, Germany). Synthesis of such proteins to

order is performed as a commercial service by many commercial peptide synthesizing service companies, e.g., Quality Controlled Biochemicals, Inc., (Hopkinton, Massachusetts); Chiron Mimotopes Peptide Systems (San Diego, California); Bachem Bioscience, Inc. (Philadelphia, Pennsylvania); Severn Biotech Ltd. (Kiddeminster, England).

Alternatively, the proteins of this invention may be produced using synthetic and recombinant nucleic acid technology. For example, one of ordinary skill in the art can design from the known genetic code a 5' to 3' nucleic acid sequence encoding a proteins of this invention. The amino acid sequence for a mature human CETP is known (SEQ ID NO: 1), as well as its corresponding DNA sequence (SEQ ID NO: 2) (see, Drayna et al., *Nature*, 327: 632 - 634 (1987)). Furthermore, the amino acid sequence for a mature rabbit CETP is known (SEQ ID NO: 3), as well as its corresponding DNA sequence (SEQ ID NO: 4) (see, Nagashima et al., *J. Lipid. Res.*, 29: 1643 - 1649 (1988)). A DNA molecule containing the coding sequences of desired CETP (or a modified "mammalianized" CETP as described above) can readily be synthesized either using an automated DNA synthesizer (e.g., Oligo 1000 DNA Synthesizer, Beckman Corp.) or by contracting with a commercial DNA synthesizing service (e.g., Genset Corp., La Jolla, California).

The synthesized or cloned DNA molecule can then be inserted into any of a variety of available gene expression systems (e.g., bacterial plasmids; bacteriophage expression vectors, retroviral expression vectors, baculoviral expression vectors), using standard methods available in the art (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, Vols. 1-3 (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989)) and/or as directed by the manufacturer of a particular commercially available gene expression system (e.g., pPROEX™-1 bacterial cell expression system; SFV eukaryotic cell expression system; CHO (Chinese Hamster Ovary cells) expression system; BAC-TO-BAC™ baculovirus expression system; Life Technologies, Inc., Gaithersburg, Maryland). Especially preferred for the expression of CETP is the CHO expression system. The expressed CETP can then be isolated from the expression system using standard methods to purify proteins.

Purification of the non-endogenous CETPs of this invention may be expedited by employing affinity chromatography or immunoprecipitation based on using antibodies to the particular CETP domain. For example, the Mab TP2 (Hesler et al., *J. Biol. Chem.*, 263: 5020-5023 (1988)) binds to the carboxyl terminal 26 amino acids of human CETP, and could be useful in one or more immunoaffinity steps in a purification scheme. Another method that

could be used in the purification of the proteins is standard column chromatography (Weinberg et al., *J. Biol. Chem.*, 269: 29588-29591 (1994)).

The non-endogenous CETPs as described herein are used to make vaccines compositions that elicit, when administered to a mammal, production of endogenous antibodies which specifically bind to endogenous CETP of the mammal and/or modulate (i.e., decrease or inhibit) endogenous CETP activity in the mammal. The anti-CETP vaccine compositions of this invention may contain one or several different non-endogenous CETPs.

In addition, the non-endogenous CETP may be linked to other molecules that may enhance the immunogenicity of the peptides.

The vaccine compositions for administration of non-endogenous CETP according to this invention can also advantageously take the form of plasmid-based vaccines for producing non-endogenous CETP *in situ*, eliciting autoantibodies directed to endogenous CETP. Such plasmid-based vaccines are, specifically, DNA plasmids which are administered (for example, by intramuscular injection or intradermal ballistic administration) to an individual. The administered DNA plasmids encode and direct the production of immunogenic non-endogenous CETP. We have discovered that such immunogenic CETP elicits the production of autoantibodies that react specifically with (i.e., bind to) endogenous CETP in the individual.

An example of a recombinant plasmid that can be used to produce a non-endogenous CETP for use according to this invention is plasmid pCMV-CETP/TT in which the CMV promoter directs transcription of a sequence encoding a vaccine peptide described in the previously mentioned PCT/US96/06147. *E. coli* bearing plasmid pCMV-CETP/TT has been deposited with the American Type Culture Collection (ATCC, Rockville, MD) and assigned Accession No. 98038. DNA coding for a desired CETP can be inserted in place of the vaccine peptide coding sequence in pCMV-CETP/TT and used for the expression of full-length CETP molecules.

The production of anti-CETP antibodies promotes a physiological state associated with a decreased risk of cardiovascular disease. The beneficial modulation of CETP activity produced by the DNA vaccines is evidenced by a significantly decreased or eliminated CETP activity; by an anti-atherogenic lipoprotein profile (for example, an increase in the level of HDL or HDL-C compared to LDL, LDL-C, VLDL, or VLDL-C); or by an inhibition

(including prevention) or decrease in the development of atherosclerotic lesions in cardiovascular tissue, such as the aorta.

General methods of administering and testing vaccines are well known to those skilled in the art (see, e.g., Talwar et al., *Proc. Natl. Acad. Sci. USA*, 91: 8532-8536 (1994)). The immune response to endogenous CETP should significantly inhibit CETP activity, alter the serum half-life of CETP, cause clearance CETP through formation of immune complexes, alter the trafficking of HDL-cholesterol, shift the equilibrium of cholesterol content of lipoproteins, alter cholesterol catabolism, and/or reduce development of atherosclerotic lesions. Control of LDL, VLDL and/or HDL levels is an accepted indicator or endpoint in treatment of cardiovascular disease, as these levels are correlated with a decreased risk of cardiovascular disease or further progression of such disease (see, e.g., Mader, in Human Biology, 4th ed., pp. 83, 102 (Wm. C. Brown Publishers, Dubuque, Iowa, 1995)). Accordingly, the desired prophylactic or therapeutic effect according to this invention is evidenced by eliciting antibodies in an individual that bind to endogenous CETP and/or inhibit endogenous CETP activity, or by a relative decrease in LDL and/or VLDL levels compared to HDL levels as the titer of antibody directed against the endogenous CETP rises, or by a decrease of absolute levels of circulating LDL and/or VLDL with the production of anti-CETP antibodies, or by an inhibition or decrease in development of atherosclerotic lesions in cardiovascular tissue.

As demonstrated herein, administration of non-endogenous CETP in a rabbit model of atherosclerosis led to a significant decrease in the development of atherosclerotic plaques. This evidence indicates that vaccination to elicit antibodies to endogenous CETP may be a useful method of treating or preventing atherosclerosis.

The successful use of non-endogenous CETP to elicit anti-endogenous CETP antibodies and to modulate the activity of native CETP was surprising in a number of respects. Previously, the use of whole CETP molecules had been avoided, since it was not known whether introduction of a whole, non-endogenous CETP molecule would provide the desired immunogenic effects. For example, non-endogenous CETP might function perfectly well as a CETP and exacerbate already undesirable cholesterol levels and metabolism. In addition, it was contemplated that full-length CETP molecules might contain immunogenic segments that would elicit antibodies capable of reacting or interfering with proteins or receptors outside of the CETP metabolic pathway, resulting in dangerous cross-reactions or

side-effects. Finally, it was not known whether introduction of a non-endogenous CETP would be able to break tolerance in the subject vaccinated, leading to production of antibodies reactive not with (or not *only* with) the non-endogenous CETP but with the *native* CETP. These uncertainties have now been resolved.

The non-endogenous CETP vaccine compositions may be administered by any route used for vaccination, including: parenterally such as intraperitoneally, interperitoneally, intradermally, subcutaneously, intramuscularly, intravenously or orally. Preferably, the vaccines of this invention are administered parenterally, e.g., intraperitoneally, interperitoneally, subcutaneously, intradermally, intramuscularly, or intravenously. If oral administration of a vaccine peptide is desired, any pharmaceutically acceptable oral excipient may be mixed with the vaccine peptides of this invention, for example, such as solutions approved for use in the oral polio vaccine. As with certain other vaccines, such as for tetanus, an occasional booster administration of the CETP vaccine compositions may be necessary to produce or maintain a desired level of modulation or inhibition of endogenous CETP. Biodegradable microspheres, such as those comprised of poly (DL-lactide-co-glycolide), have been shown to be useful for effective vaccine delivery and immunization via oral or parenteral routes.

Appropriate dosages of the non-endogenous CETP may be established by general vaccine methodologies used in the art based on measurable parameters for which the vaccine is proposed to affect, including monitoring for potential contraindications, such as hypersensitivity reaction, erythema, induration, tenderness (see, e.g., Physician's Desk Reference, 49th ed., (Medical Economics Data Production Co., Mont Vale, New Jersey, 1995), pp. 1628, 2371 (referring to hepatitis B vaccine), pp. 1501, 1573, 1575 (referring to measles, mumps, and/or rubella vaccines), pp. 904, 919, 1247, 1257, 1289, 1293, 2363 (referring to diphtheria, tetanus and/or pertussis vaccines)) ; Talwar, G.P., et al., *Proc. Natl. Acad. Sci. USA, 91*: 8532-8536 (1994)). A common and traditional approach for vaccinating humans is to administer an initial dose of a particular vaccine to sensitize the immune system and then follow-up by one or more "booster" doses of the vaccine to elicit an anamnestic response by the immune system that was sensitized by the initial administration of the vaccine (vaccination). Such a "primary and booster" administration procedure has been well known and commonly used in the art, as for example, in developing and using measles, polio, tetanus, diphtheria, and hepatitis B vaccines.

Initially, the amount of a vaccine composition administered to an individual may be that required to neutralize the approximate level of endogenous CETP activity present in the individual prior to vaccination, as can be determined by measuring CETP activity in serum or plasma samples from the individual, for example as determined using a commercially available CETP assay (e.g., Roar Biomedical., Yonkers, New York). Plasma or serum samples from a vaccinated individual can also be monitored to determine whether a measurable increase in the levels of total HDL or HDL-C is seen after administration of the non-endogenous CETP using commercially available assays (e.g., available from Sigma Diagnostics, Inc., Saint Louis, Missouri). A rise in the concentration (titer) of circulating anti-CETP antibodies can be measured in plasma or serum samples, for example using an ELISA assay. Thus, it is possible and recommended that initially it be established whether a rise in anti-CETP antibody can be correlated with an increase in the level of HDL or HDL-C, a decrease in LDL or VLDL, or with a decrease in CETP activity. Thereafter, one need only monitor a rise in titer of anti-CETP antibody to determine whether a sufficient dosage of vaccine peptide has been administered or whether a "booster" dose is indicated to elicit an elevated level of anti-CETP antibody. This is the common procedure with various established vaccinations, such as vaccination against hepatitis B virus.

Three-dimensional arterial imaging methods are currently available which can be used to identify arterial lesions and monitor their development or regression in an individual (see, for example, McPherson, *Scientific American Science & Medicine,* pages 22-31, (March/April, 1996)). Thus such imaging methods can be used to monitor the effectiveness of vaccination according to the methods of this invention.

A more complete appreciation of this invention and the advantages thereof can be obtained from the following non-limiting examples.

EXAMPLE 1

Immunization of Rabbits Against Endogenous CETP

Four vaccine preparations were made for injection into four groups of twelve New Zealand White Rabbits, to test the ability of the vaccine preparation to elicit an immune response against endogenous rabbit CETP. Group I (negative control) contained rabbits #1 - #12, each of which was injected with a vaccine composition containing an irrelevant antigen, human chorionic gonadotropin (hCG). Group II (comparative embodiment of

PCT/US96/06147) contained rabbits #13 - #24, each of which was injected with a vaccine peptide having a portion of the C-terminus of human CETP and a portion of tetanus toxoid ("Peptide"; see, SEQ ID NO: 7). Group III (this invention) contained rabbits #25 - #36, each of which received a vaccine composition containing whole recombinant human CETP ("rhuCETP"). Group IV (this invention) contained rabbits #37 - #48, each of which received a vaccine composition containing whole recombinant human CETP conjugated with whole tetanus toxoid using a chemical crosslinker ("Conjugate").

The general protocol for vaccinating and testing the rabbits was as follows: On Day 1, each rabbit received one subcutaneous injection of a composition containing 200 μg of immunogen in Complete Freund's Adjuvant (Sigma Chemical Co., St. Louis, Missouri). Each composition suspended the respective immunogen in phosphate buffered saline (PBS) and emulsified with complete Freund's adjuvant (1:1) to yield a final concentration of 100 μg/100 μl. Each rabbit was administered the vaccine mixture in a 200 μl dose (200 μg immunogen) at one subcutaneous site. A boost of 200 μg of immunogen in Incomplete Freund's Adjuvant (Sigma Chemical Co.) was administered as on Day 1 at Day 28 and Day 49. Blood samples (approximately 1-5 ml) were withdrawn from the ear of each rabbit (prior to injections) on Days 1 ("prebleed"), 28 , 49, 105, 147 and 217. Blood plasma samples were prepared by standard centrifugation methods to separate cellular components from the plasma. Plasma samples were stored at -70° C. Plasma samples of both Groups I and II were analyzed for presence of and increase in titer of anti-CETP antibodies and for CETP activity, CETP mass, and plasma levels of various lipoprotein components (HDL, LDL, triglycerides).

Direct ELISA for Titering Anti-CETP Antibodies

A sandwich enzyme-linked immunosorbent assay (ELISA) was used to titer plasma samples containing anti-CETP antibody. A biotinylated C-terminal peptide (20 amino acids) of rabbit CETP was adsorbed to wells of a microtiter dish coated with streptavidin, and various dilutions of rabbit plasma from the rabbits of Groups I - III were added to each well. Non-specific binding can be blocked by adding a 1% solution of BSA in PBS and 0.05% Tween to each well and incubating for 2 hours at room temperature (20 -22° C) on a rotating shaker at 150 rpm. The wells were then washed four times with ELISA wash buffer (PBS + 0.05% Tween). Plasma samples were then diluted in dilution buffer (e.g.1% BSA in PBS), followed by serial dilutions in the same buffer. Diluted samples (100 μl) were added to the wells, incubated for 1.5-2 hours at room temperature on a rotating shaker at 150 rpm, and

then washed 4 times with ELISA wash buffer (PBS + 0.05% Tween). To detect bound anti-CETP antibodies, 100 µl of an optimized dilution of horseradish peroxidase (HRP) labeled goat anti-rabbit immunoglobulin (Southern Biotechnology Associates, Inc.; Birmingham, Alabama; or Jackson Immunoresearch, Inc.; West Grove, Pennsylvania) in dilution buffer was added, and the plates were incubated for 2 hours at room temperature on a rotating shaker at 150 rpm. The wells were then washed four times with ELISA wash buffer (see above), peroxidase substrate TMB (TMB peroxidase substrate, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland) was added, and the plates were incubated 30 minutes at room temperature. Change in optical density was monitored spectrophotometrically at 450 nm using an ELISA reader (e.g., E-max, Molecular Device Corp., Menlo Park, California). In this assay, the O.D. was directly proportional to the amount of anti-CETP antibodies present in the plasma samples.

The results of the assay for each of the rabbit groups is shown in Figure 2. Group I showed no plasma antibodies detecting the rabbit C-terminal CETP peptide, whereas the groups vaccinated with CETP immunogens (Groups II, III) showed significant titers of anti-CETP antibodies in almost all vaccinated rabbits. Analysis of the Group IV data is in progress.

CETP Activity and Neutralization Assays

In order to measure the activity of CETP in plasma, a commercial fluorescence-based assay (Roar Biomedical Inc.; Yonkers, New York) was used. Incubation of a CETP source (rhuCETP, huCETP or rabbit CETP) with the donor and acceptor particles, included in the kit, results in the CETP-mediated transfer of a fluorescent neutral lipid. This fluorescent neutral lipid is present in a self-quenched state when contained within the core of the donor. The CETP-mediated transfer is determined by the increase in fluorescence intensity as the fluorescent neutral lipid is removed from the self-quenched donor to the acceptor. To measure neutralization, anti-CETP antibodies are isolated from the plasma of vaccinated rabbits with protein A. Identical amounts (measured by A_{280}) of the antibodies from various samples are added to the above reaction.

Figure 3 shows inhibition of CETP activity by rabbit antibodies collected from the plasma of the vaccinated rabbits. The bar designated "Positive Control Mab" refers to anti-CETP monoclonal antibody TP2, which is known to inhibit CETP activity in vitro and is

included for comparison. Figure 4 shows the change in CETP activity in Groups I and III from week 1 to week 32. Analysis of the Group IV data is in progress.

Cholesterol and HDL Levels in Plasma Samples of Vaccinated Rabbits

The plasma samples taken from rabbits of Groups I - IV were also assayed for the concentration of total cholesterol (Figure 5), HDL-C (Figure 6), and triglycerides (see Figure 8) using standard commercial assays (Sigma Diagnostics, Inc., Saint Louis, Missouri). LDL-C (Figure 7) is calculated as total cholesterol minus HDL-C minus 0.2 × tryglyceride level. CETP levels were determined by a slot blot analysis using anti-CETP monoclonal antibody TP2 and chemiluminescences for detection. The band intensities obtained with various amounts of plasma samples were quantified with the aid of a Kodak® DC40 camera and 1D Image Analysis software (version 1.6), then compared to that obtained with known amounts of purified human CETP loaded on the same nitrocellulose filter.

The plasma lipoprotein profile for the Group III rabbit #32, which showed the most pronounced reduction in LDL-C levels (Fig. 7) is shown in Figure 8. The profile shows a dramatic rise in HDL as a percent of the total lipoprotein profile. Figure 9 shows, for this rabbit, the correlation between decreasing CETP activity, decreasing cholesterol mass and increasing HDL as a percentage of total lipoportein.

Measurement of cholestrol deposits in the irises of vaccinated rabbits

The rabbits from Groups I - IV were also assayed for the amount of cholestrol deposits detected in the irises. A scale of cholestrol deposition in the iris was established, with 0 = no deposit, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80%, and 5 = 100% deposits on this iris (i.e., iris completely covered with deposits). One iris per rabbit was evaluated and scored for degree of cholestrol deposition. The groups of animals were blinded to the scorer to avoid bias. Figure 10 shows the data collected from all 48 animals. The Xs indicate animals for which no data were obtained (rabbit #34, #39, and #42); these animals were euthanized due to unrelated complications such as furballs. The data indicate that all CETP vaccinated groups had statistically less cholestrol deposits than the control group.

Quantitation of lesions in aorta of vaccinated rabbits

The rabbits were switched from a diet of basic rabbit chow to diets supplemented with 0.25% cholesterol (w/w) known to produce atherosclerotic-like lesions in rabbits (Daley et al., *Arterioscler. Thromb., 14*: 95 - 104 (1994)). To determine whether the vaccination may affect the development of atherosclerosis, the aortas of these rabbits were examined for the

development of atherosclerotic lesions. After blood samples were taken on the last day, rabbits were sacrificed. The entire aortas from each of Groups I-IV were removed and placed into fixative solution (3.7 % v/v formaldehyde). Loose tissue, adherent fat, and the adventitia were dissected free from the arteries. Each artery was then cut lengthwise, pinned flat to expose the intimal (luminal) surface, stained with Sudan IV, and then photographed. Sudan IV is a fat soluble red dye that stains atherosclerotic plaques on the intimal surface of arteries. Figure 11 summarizes the results of this experiment. The stained aortas of rabbits vaccinated with human chorionic gonadotropin ("HCG") revealed a prevalence of atherosclerotic lesions along the length of the aortas and particularly in the portion of the aortas from the thoracic region. In contrast, the aortas of rabbits vaccinated with a synthetic vaccine peptide having segments of tetanus toxoid and the C-terminal sequence of human CETP ("Peptide", see SEQ ID NO: 7), full-length recombinant human CETP ("rhuCETP"), and a CETP-tetanus toxoid conjugate composition ("Conjugate") had lower incidence of lesions, including the portion of the aorta from the thoracic region.

To quantitate the noticeable difference in the presence of atherosclerotic lesions in the aortas of rabbits or lack thereof, the total surface area of the pinned aortas and that of the aortic lesions was determined from photographs by planar morphometry (Daley et al., 1994) using a digitizing tablet with associated software (THE MORPHOMETER™, Woods Hole Educational Associates, Woods Hole, Massachusetts). The percentage of the surface area of the aortas covered by lesions was determined and the percentages are represented in Figure 11.

EXAMPLES 2 and 3

Plasmid-based Vaccines in Mice and Rabbits

Four groups of mice were vaccinated intramuscularly with one of the following:

1. pCMV: a plasmid vector having the cytomegalovirus immediate early promoter/enhancer but without any operably-linked structural gene
2. pCIII-huCETP: a plasmid vector having the full coding sequence for human CETP (SEQ ID NO: 1) under the transcriptional control of the human Apo CIII promoter
3. pSV40-huCETP: a plasmid vector having the full coding sequence for human CETP under the transcriptional control of the SV40 promoter

4. pCMV-TT-rabCETP: a plasmid vector having a tetanus toxoid peptide (amino acid 2 to 15 of SEQ ID NO: 7) coding sequence and the full coding sequence for rabbit CETP (SEQ ID NO: 3) under the transcriptional control of the cytomegalovirus immediate early promoter/enhancer

The mice were injected once with 25 µl of PBS containing 100 µg of the plasmid and blood samples were periodically collected and were analyzed with an ELISA detecting anti-human CETP antibodies with the method described below:

Plastic 96-well microtiter plates were coated with Protein A/G, by incubating 100 µl of a 5 µg/ml PBS solution per well overnight at 4°C. The plates were emptied and the wells were blocked with 200 µl of blocking buffer (PBS with 4% BSA, 1% sucrose, 0.5% NP-40, 0.01% Gentamycin) for 2 to 8 hours at room temperature. Antibodies from the plasma samples were captured on the Protein A/G by incubating 100 µl of various dilutions of the samples for 1 hour at room temperature. Following washing of the wells, biotinylated CETP was captured by the plate bound antibodies by incubating 100 µl of a biotinylated CETP solution at room temperature for 1 hour. The bound CETP was detected by incubating 100 µl of a streptavidin-HRP solution for 30 minutes at room temperature, followed by adding 100 µl of substrate, stopping the reaction with 0.18M sulfuric acid and reading the optical density at 450 nm. Figure 12 shows that plasmids delivered to Groups 2, 3, and 4 produce immunogenic xenogeneic protein.

Subsequently, rabbits were vaccinated with 300 µg (equally split in six intramuscular sites in the quadriceps) of a vector carrying the human CETP coding sequence under the transcriptional control of the SV40 promoter enhancer (SV40-huCETP) or the same vector without the CETP coding sequence (SV40). The primary injection occurred on Day 1 with an identical boost on weeks 5, 8, 26, and 30. Blood samples were taken periodically throughout the experiment and animals were terminated on week 34.

The plasma samples from the vaccinated rabbits were subjected to the ELISA for detection of antibodies to whole recombinant human CETP, as described above. Figure 13A-K summarizes the titration of the antibody measured in the rabbits vaccinated with the SV40 promoter enhancer (SV40-huCETP) between weeks 1-34. Significant antibody production was detected on weeks 30 and 34, depicted by the open diamond and open square symbols of the graphs, in most animals. Two of the 11 rabbits (Figure 13A, and 13E) were non-responders.

The rabbits were switched from a diet of basic rabbit chow to diets supplemented with 0.25% cholesterol (w/w) known to produce atherosclerotic-like lesions in rabbits. The lesions in rabbits vaccinated with pSV40-huCETP and control rabbits vaccinated with pSV40 were visualized and quantitated as described in Example 1 above. Figure 14 shows the mean percentage of aorta covered with lesions in both groups of rabbits. The results of this experiment are in line with the trend observed with directly vaccinated rabbits (Groups I-IV, see Figure 11), showing a decrease in aortic lesions due to vaccination.

Although a number of embodiments have been described above, it will be understood by those skilled in the art that modifications and variations of the described compositions and methods may be made without departing from either the spirit of the invention or the scope of the appended claims. The articles and publications cited herein are incorporated by reference.

CLAIMS:

1. A vaccine composition effective to promote production of antibodies binding to endogenous cholesteryl ester transfer protein (CETP) in a mammal, consisting essentially of a non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

2. A vaccine composition as defined in Claim 1, wherein said mammal is a human and said non-endogenous CETP is selected from the group consisting of rabbit CETP, mouse CETP, simian CETP, humanized rabbit, mouse or simian CETP, and allelic variants or polymorphs of said human's CETP.

3. A vaccine composition effective to promote production of antibodies binding to endogenous cholesteryl ester transfer protein (CETP) in a mammal, consisting essentially of a mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

4. A vaccine composition as defined in Claim 3, wherein said mammalianized CETP is a humanized rabbit CETP.

5. A vaccine composition as defined in Claim 4, wherein said humanized rabbit CETP has the amino acid sequence of SEQ ID NO. 5.

6. A vaccine composition as defined in Claim 1, wherein said mammal is a rabbit and said non-endogenous CETP is human CETP.

7. A vaccine composition as defined in Claim 1 or 3, which contains an adjuvant selected from the group consisting of alum, Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, RIBI Adjuvant System.

8. A plasmid-based vaccine comprising a promoter sequence suitable for directing the transcription of a nucleotide sequence in a cell of a mammal operably linked to a nucleotide

sequence coding for a cholesteryl ester transfer protein (CETP) that is non-endogenous to said mammal.

9. A plasmid-based vaccine as defined in Claim 8, wherein said mammal is a human and said non-endogenous CETP is selected from the group consisting of rabbit CETP, mouse CETP, simian CETP, humanized rabbit, mouse or simian CETP, and allelic variants or polymorphs of said human's CETP.

10. A plasmid-based vaccine as defined in Claim 8, wherein said mammal is a human and said non-endogenous CETP is rabbit CETP.

11. A plasmid-based vaccine as defined in Claim 8, wherein said mammal is a human and said non-endogenous CETP is a humanized rabbit CETP.

12. A plasmid-based vaccine as defined in Claim 11, wherein said non-endogenous CETP has the amino acid sequence of SEQ ID NO: 5.

13. A plasmid-based vaccine as defined in Claim 8, wherein said mammal is a rabbit and said non-endogenous CETP is a human CETP.

14. A plasmid-based vaccine as defined in Claim 8, wherein the promoter is the cytomegalovirus immediate early promoter/enhancer.

15. A method for promoting production in a mammal of antibodies binding the mammal's endogenous CETP comprising administering to the mammal a non-endogenous CETP or a mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

16. A method for elevating the ratio of circulating high density lipoprotein-associated cholesterol to circulating low density lipoprotein-associated cholesterol or total cholesterol in a mammal comprising administering to the mammal a non-endogenous CETP or a

mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

17. A method for decreasing the level of endogenous CETP activity in a human or other animal comprising administering to the mammal a non-endogenous CETP or a mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

18. A method for altering the metabolism of low density lipoprotein-associated cholesterol to decrease the development of atherosclerotic lesions in a mammal comprising administering to the mammal a non-endogenous CETP or a mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

19. A method of lowering the level of circulating low density lipoprotein in a mammal comprising administering to the mammal a non-endogenous CETP or a mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

20. A method of lowering the level of total circulating cholesterol in a mammal comprising administering to the mammal a non-endogenous CETP or a mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

21. A method for therapeutically or prophylactically treating atherosclerosis in a mammal comprising administering to the mammal a non-endogenous CETP or a mammalianized non-endogenous CETP, optionally in combination with an adjuvant effective to non-specifically stimulate the immune response of said mammal.

22. A method according to any of Claims 15-21, wherein the mammal is a human and the non-endogenous CETP is a rabbit CETP.

23. A method according to any of Claims 15-21, wherein said mammalianized CETP is a humanized rabbit CETP.

24. A method according to Claim 23, wherein said humanized rabbit CETP has the amino acid sequence of SEQ ID NO. 5.

25. A method according to any of Claims 15-21, wherein said CETP is administered in combination with an adjuvant selected from the group consisting of alum, Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, RIBI Adjuvant System.

26. A method for promoting production in a mammal of antibodies reactive with the mammal's endogenous CETP comprising transfecting at least one cell of the mammal with a plasmid-based vaccine according to Claim 8.

27. A method for elevating the ratio of circulating high density lipoprotein-associated cholesterol to circulating low density lipoprotein-associated cholesterol or total cholesterol in a mammal comprising transfecting at least one cell of the mammal with a plasmid-based vaccine according to Claim 8.

28. A method for decreasing the level of endogenous CETP activity in a human or other animal comprising transfecting at least one cell of the mammal with a plasmid-based vaccine according to Claim 8.

29. A method for altering the metabolism of low density lipoprotein-associated cholesterol to decrease the development of atherosclerotic lesions in a mammal comprising transfecting at least one cell of the mammal with a plasmid-based vaccine according to Claim 8.

30. A method of lowering the level of circulating low density lipoprotein in a mammal comprising transfecting at least one cell of the mammal with a plasmid-based vaccine according to Claim 8.

31. A method of lowering the level of total circulating cholesterol in a mammal comprising transfecting at least one cell of the mammal with a plasmid-based vaccine according to Claim 8.

32. A method for therapeutically or prophylactically treating atherosclerosis in a mammal comprising transfecting at least one cell of the mammal with a plasmid-based vaccine according to Claim 8.

33. A method according to any of Claims 26-32, wherein the mammal is a human and the plasmid-based vaccine includes nucleic acid encoding a rabbit CETP.

34. A method according to any of Claims 26-32, wherein the mammal is a human and the plasmid-based vaccine includes nucleic acid encoding a humanized rabbit CETP.

35. A method according to Claims 34, wherein said humanized rabbit CETP has the amino acid sequence of SEQ ID NO. 5.

36. A method according to any of Claims 26-32, which comprises the additional step of administering to said mammal an adjuvant effective to non-specifically stimulate the immune response of said mammal.

37. A method according to Claim 36, wherein the adjuvant is selected from the group consisting of alum, Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, RIBI Adjuvant System.

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Cys Pro Lys Gly Ala Ser Tyr Glu Ala Gly Ile Val Cys Arg Ile Thr Lys Pro Ala Leu Leu Val Leu Asn Gln  25
 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
Cys Ser Lys Gly Thr Ser His Glu Ala Gly Ile Val Cys Arg Ile Thr Lys Pro Ala Leu Leu Val Leu Asn His

Glu Thr Ala Lys Val Val Gln Thr Ala Phe Gln Arg Ala Gly Tyr Pro Asp Val Ser Gly Glu Arg Ala Val Met  50
 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
Glu Thr Ala Lys Val Ile Gln Thr Ala Phe Gln Arg Ala Ser Tyr Pro Asp Ile Thr Gly Glu Lys Ala Met Met

Leu Leu Gly Arg Val Lys Tyr Gly Leu His Asn Leu Gln Ile Ser His Leu Ser Ile Ala Ser Ser Gln Val Glu  75
 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
Leu Leu Gly Gln Val Lys Tyr Gly Leu His Asn Ile Gln Ile Ser His Leu Ser Ile Ala Ser Ser Gln Val Glu

Leu Val Asp Ala Lys Thr Ile Asp Val Ala Ile Gln Asn Val Ser Val Val Phe Lys Gly Thr Leu Asn Tyr Ser 100
 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
Leu Val Glu Ala Lys Ser Ile Asp Val Ser Ile Gln Asn Val Ser Val Val Phe Lys Gly Thr Leu Lys Tyr Gly

Tyr Thr Ser Ala Trp Gly Leu Gly Ile Asn Gln Ser Val Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile 125
 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
Tyr Thr Thr Ala Trp Trp Leu Gly Ile Asp Gln Ser Ile Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile

Asn Thr Glu Leu Thr Cys Asp Ala Gly Ser Val Arg Thr Asn Ala Pro Asp Cys Tyr Leu Ala Phe His Lys Leu 150
 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
Asn Thr Gln Leu Thr Cys Asp Ser Gly Arg Val Arg Thr Asp Ala Pro Asp Cys Tyr Leu Ser Phe His Lys Leu

Leu Leu His Leu Gln Gly Glu Arg Glu Pro Gly Trp Leu Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu 175
 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
Leu Leu His Leu Gln Gly Glu Arg Glu Pro Gly Trp Ile Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu
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FIG. 1A

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Lys Leu Ile Leu Lys Arg Gln Val Cys Asn Glu Ile Asn Thr Ile Ser Asn Ile Met Ala Asp Phe Val Gln Thr  200
Lys Leu Val Leu Lys Gly Gln Ile Cys Lys Glu Ile Asn Val Ile Ser Asn Ile Met Ala Asp Phe Val Gln Thr

Arg Ala Ala Ser Ile Leu Ser Asp Gly Asp Ile Gly Val Asp Ile Ser Val Thr Gly Ala Pro Val Ile Thr Ala  225
Arg Ala Ala Ser Ile Leu Ser Asp Gly Asp Ile Gly Val Asp Ile Ser Leu Thr Gly Asp Pro Val Ile Thr Ala

Thr Tyr Leu Glu Ser His His Lys Gly His Phe Thr His Lys Asn Val Ser Glu Ala Phe Pro Leu Arg Ala Phe  250
Ser Tyr Leu Glu Ser His His Lys Gly His Phe Ile Tyr Lys Asn Val Ser Glu Asp Leu Pro Leu Pro Thr Phe

Pro Pro Gly Leu Leu Gly Asp Ser Arg Met Leu Tyr Phe Trp Phe Ser Asp Gln Val Leu Asn Ser Leu Ala Arg  275
Ser Pro Thr Leu Leu Gly Asp Ser Arg Met Leu Tyr Phe Trp Phe Ser Glu Arg Val Phe His Ser Leu Ala Lys

Ala Ala Phe Gln Glu Gly Arg Leu Val Leu Ser Leu Thr Gly Asp Glu Phe Lys Lys Val Leu Glu Thr Gln Gly  300
Val Ala Phe Gln Asp Gly Arg Leu Met Leu Ser Leu Met Gly Asp Glu Phe Lys Ala Val Leu Glu Thr Trp Gly

Phe Asp Thr Asn Gln Glu Ile Phe Gln Glu Leu Ser Arg Gly Leu Pro Thr Gly Gln Ala Gln Val Ala Val His  325
Phe Asn Thr Asn Gln Glu Ile Phe Gln Glu Val Val Gly Gly Phe Pro Ser-----Gln Ala Gln Val Thr Val His

Cys Leu Lys Val Pro Lys Ile Ser Cys Gln Asn Arg Gly Val Val Val Ser Ser Ser Val Ala Val Thr Phe Arg  350
Cys Leu Lys Met Pro Lys Ile Ser Cys Gln Asn Lys Gly Val Val Val Asn Ser Ser Val Met Val Lys Phe Leu
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FIG. 1B

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Phe Pro Arg Pro Asp Gly Arg Glu Ala Val Ala Tyr Arg Phe Glu Glu Asp Ile Ile Thr Thr Val Gln Ala Ser  375
 |   |   |   |   |                   |   |   |       |   |   |   |   |       |   |   |   |   |   |
Phe Pro Arg Pro Asp Gln Gln His Ser Val Ala Tyr Thr Phe Glu Glu Asp Ile Val Thr Thr Val Gln Vla Ser

Tyr Ser Gln Lys Lys Leu Phe Leu His Leu Leu Asp Phe Gln Cys Val Pro Ala Ser Gly Arg Ala Gly Ser Ser  400
 |   |       |   |   |   |   |       |   |   |   |   |           |
Tyr Ser Lys Lys Lys Leu Phe Leu Ser Leu Leu Asp Phe Gln Ile Thr Pro------------------------------

Ala Asn Leu Ser Val Ala Leu Arg Thr Glu Ala Lys Ala Val Ser Asn Leu Thr Glu Ser Arg Ser Glu Ser Leu  425
                                              |       |   |   |   |   |   |   |       |   |   |   |
----------------------------------------------Lys Thr Val Ser Asn Leu Thr Glu Ser Ser Ser Glu Ser Ile

Gln Ser Ser Leu Arg Ser Leu Ile Ala Thr Val Gly Ile Pro Glu Val Met Ser Arg Leu Glu Val Ala Phe Thr  450
 |   |       |       |       |           |   |   |   |   |   |   |   |   |   |   |   |   |       |   |
Gln Ser Phe Leu Gln Ser Met Ile Thr Ala Val Gly Ile Pro Glu Val Met Ser Arg Leu Glu Val Val Phe Thr

Ala Leu Met Asn Ser Lys Gly Leu Asp Leu Phe Glu Ile Ile Asn Pro Glu Ile Ile Thr Leu Asp Gly Cys Leu  475
 |   |   |   |   |   |   |           |   |       |   |   |   |   |   |   |   |       |   |   |       |
Ala Leu Met Asn Ser Lys Gly Val Ser Leu Phe Asp Ile Ile Asn Pro Glu Ile Ile Thr Arg Asp Gly Phe Leu

Leu Leu Gln Met Asp Phe Gly Phe Pro Lys His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser  (SEQ ID NO: 3)  496
 |   |   |   |   |   |   |   |   |       |   |   |   |   |   |   |   |   |   |   |
Leu Leu Gln Met Asp Phe Gly Phe Pro Glu His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser  (SEQ ID NO: 1)
```

FIG. 1C

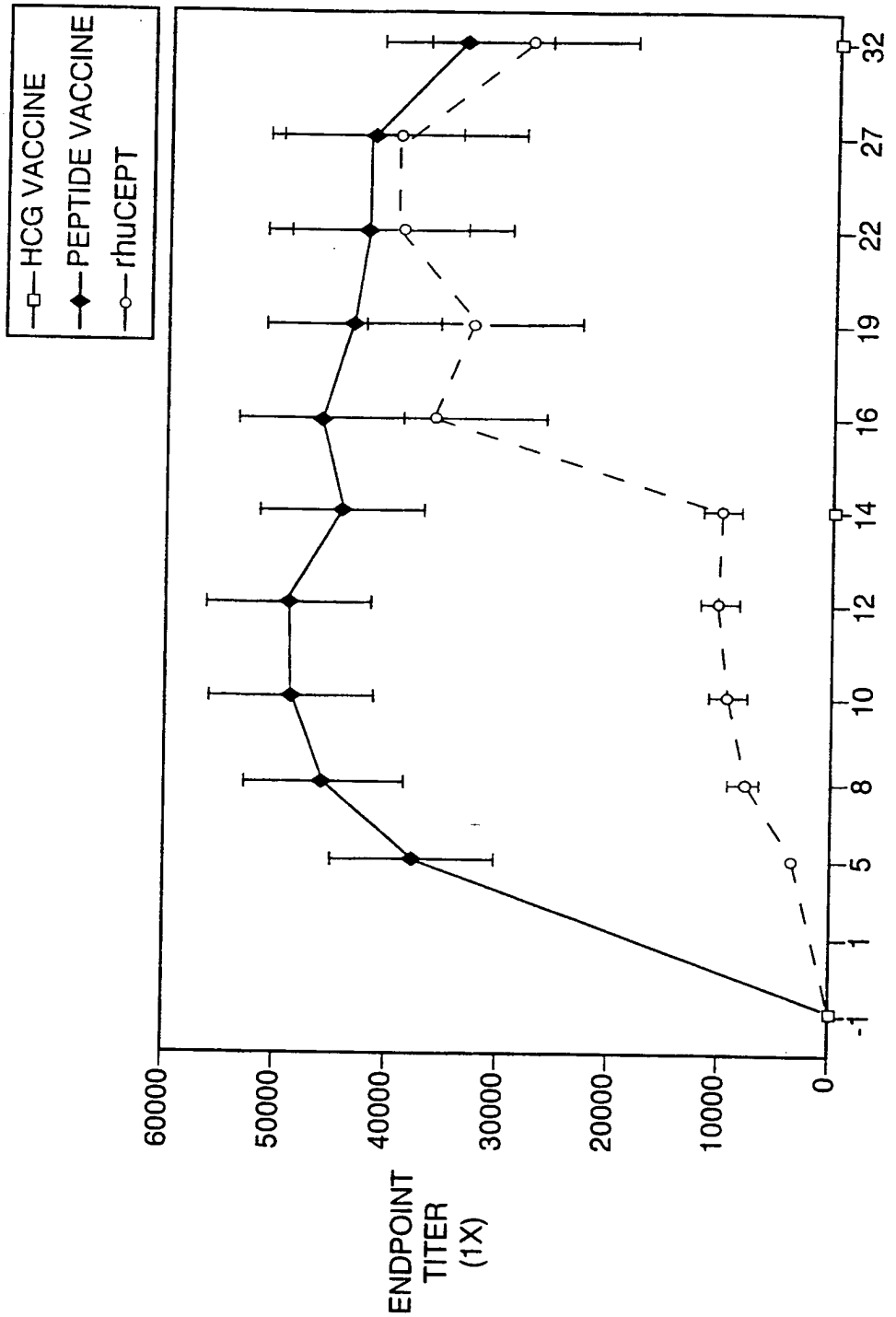

HCG VACCINE
PEPTIDE VACCINE
rhuCEPT
ENDPOINT TITER (1X)
60000
50000
40000
30000
20000
10000
0
-1
1
5
8
10
12
14
16
19
22
27
32
WEEKS

FIG. 2

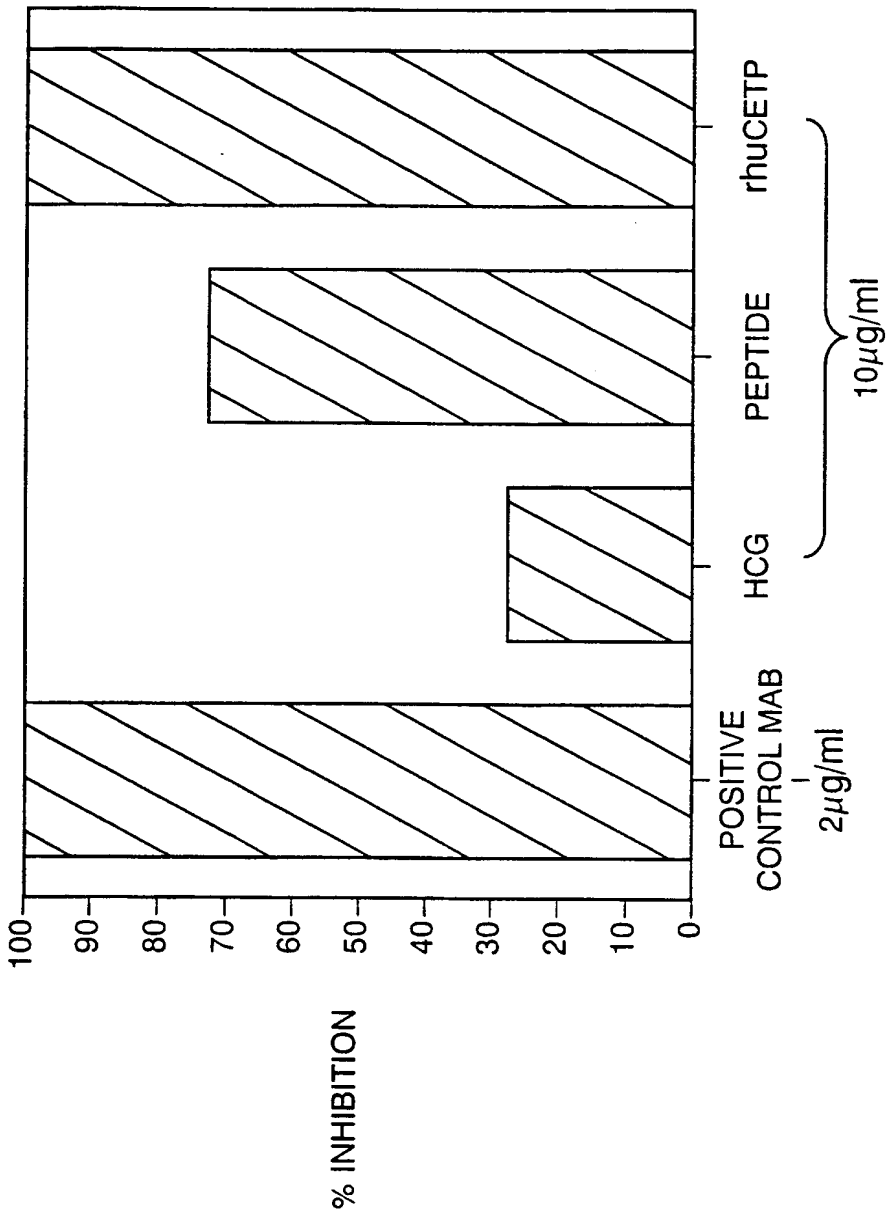

% INHIBITION
100
90
80
70
60
50
40
30
20
10
0
POSITIVE CONTROL MAB
2μg/ml
HCG
PEPTIDE
rhuCETP
10μg/ml

FIG. 3

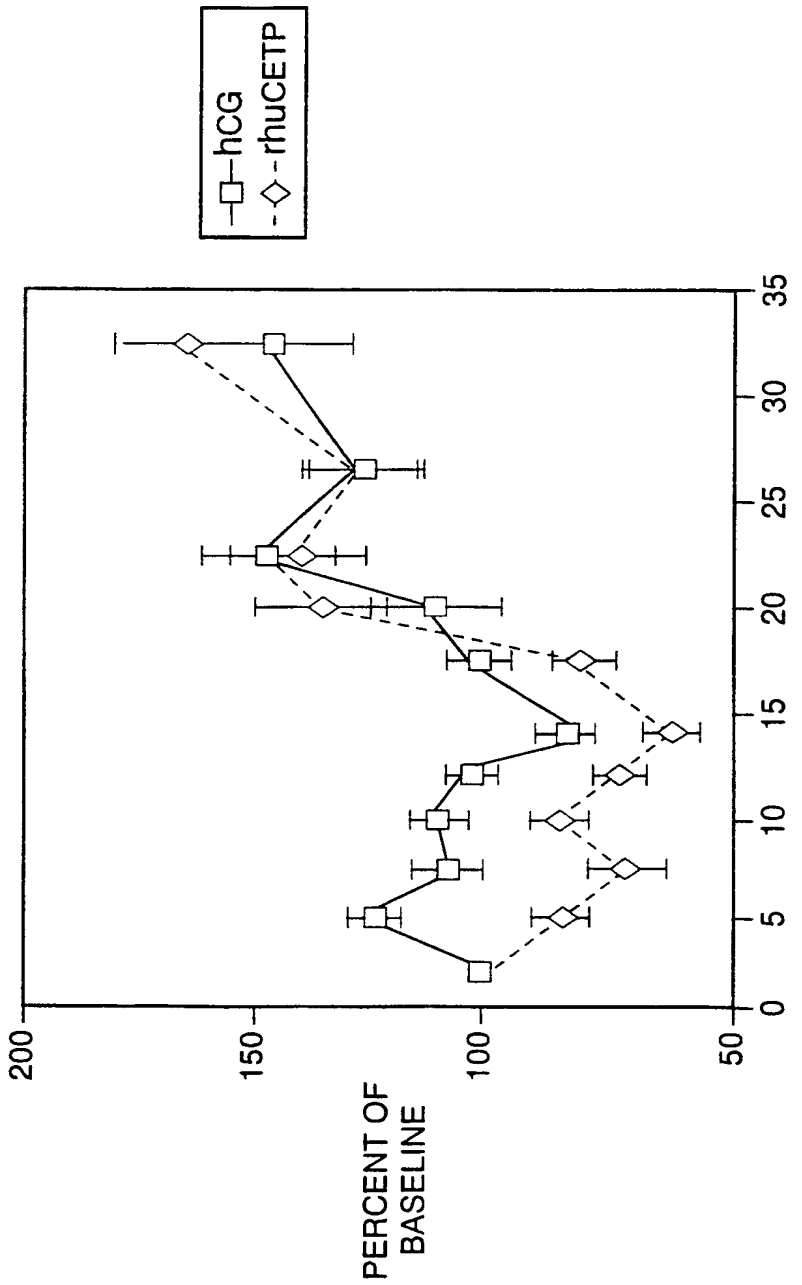

hCG
rhuCETP
PERCENT OF BASELINE
200
150
100
50
0
5
10
15
20
25
30
35
WEEKS

FIG. 4

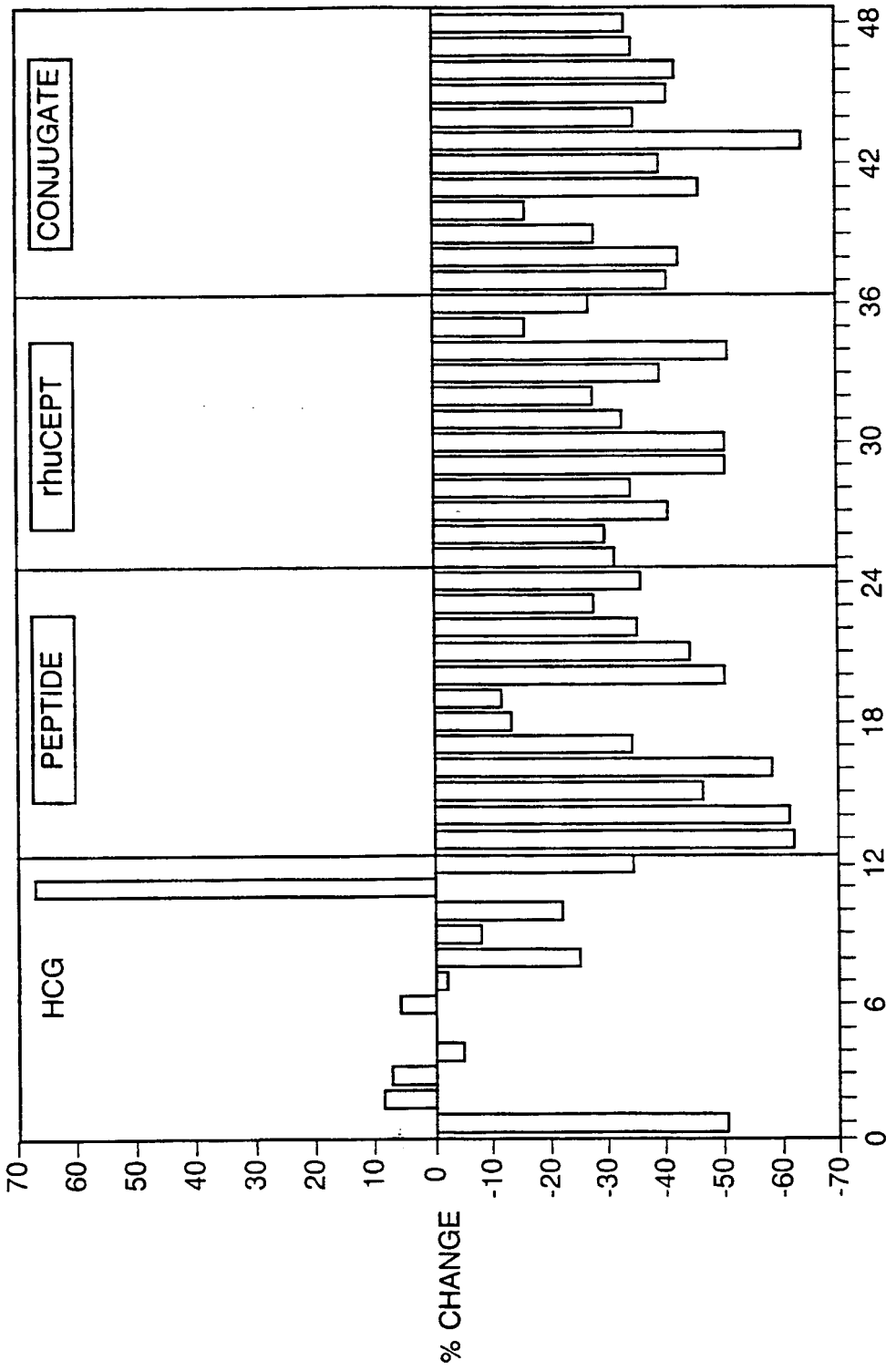

HCG
PEPTIDE
rhuCEPT
CONJUGATE
% CHANGE
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
0
6
12
18
24
30
36
42
48
RABBIT #

FIG. 5

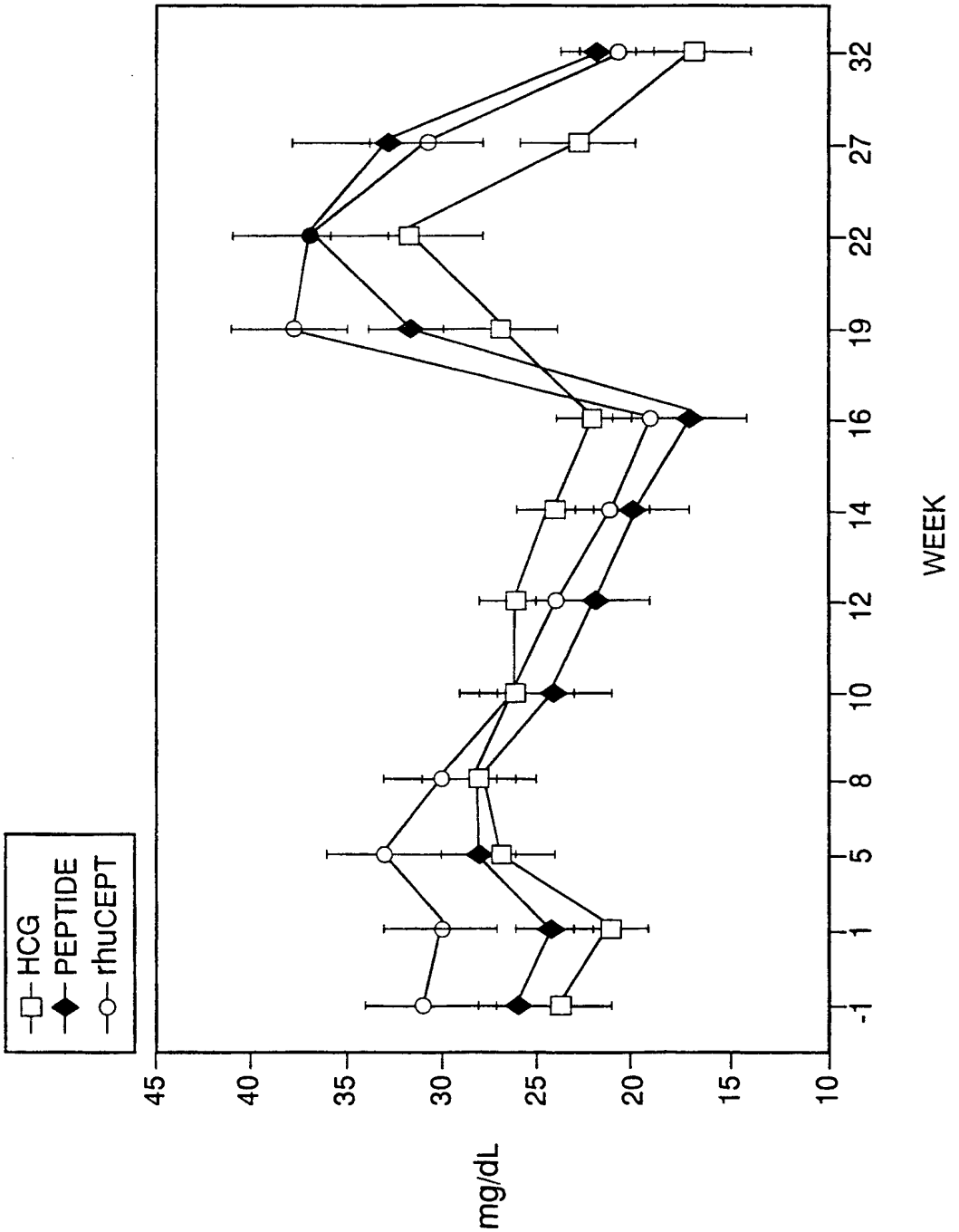

HCG
PEPTIDE
rhuCEPT
mg/dL
45
40
35
30
25
20
15
10
-1
1
5
8
10
12
14
16
19
22
27
32
WEEK

FIG. 6

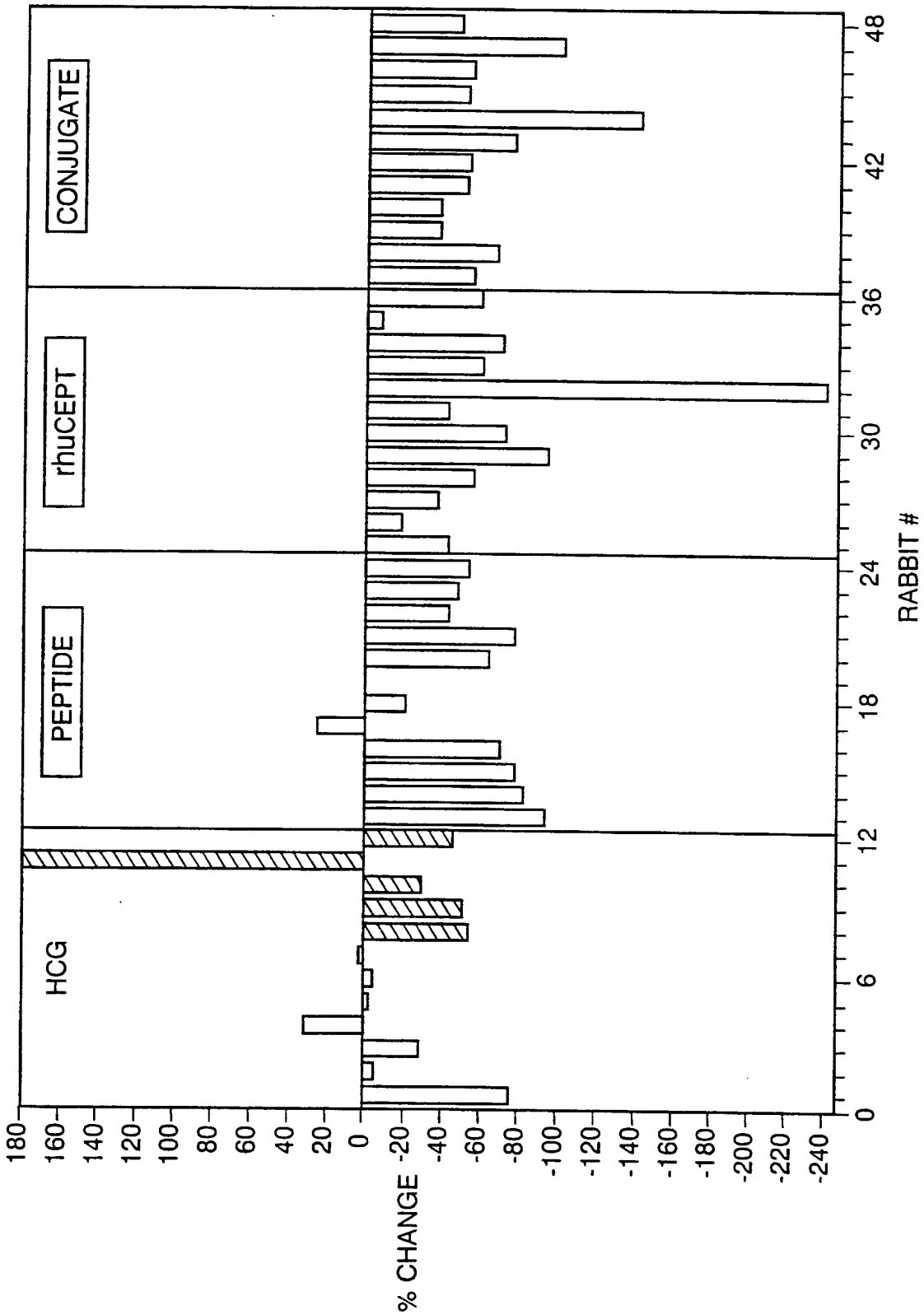

HCG
PEPTIDE
rhuCEPT
CONJUGATE
% CHANGE
RABBIT #
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
0
6
12
18
24
30
36
42
48

FIG. 7

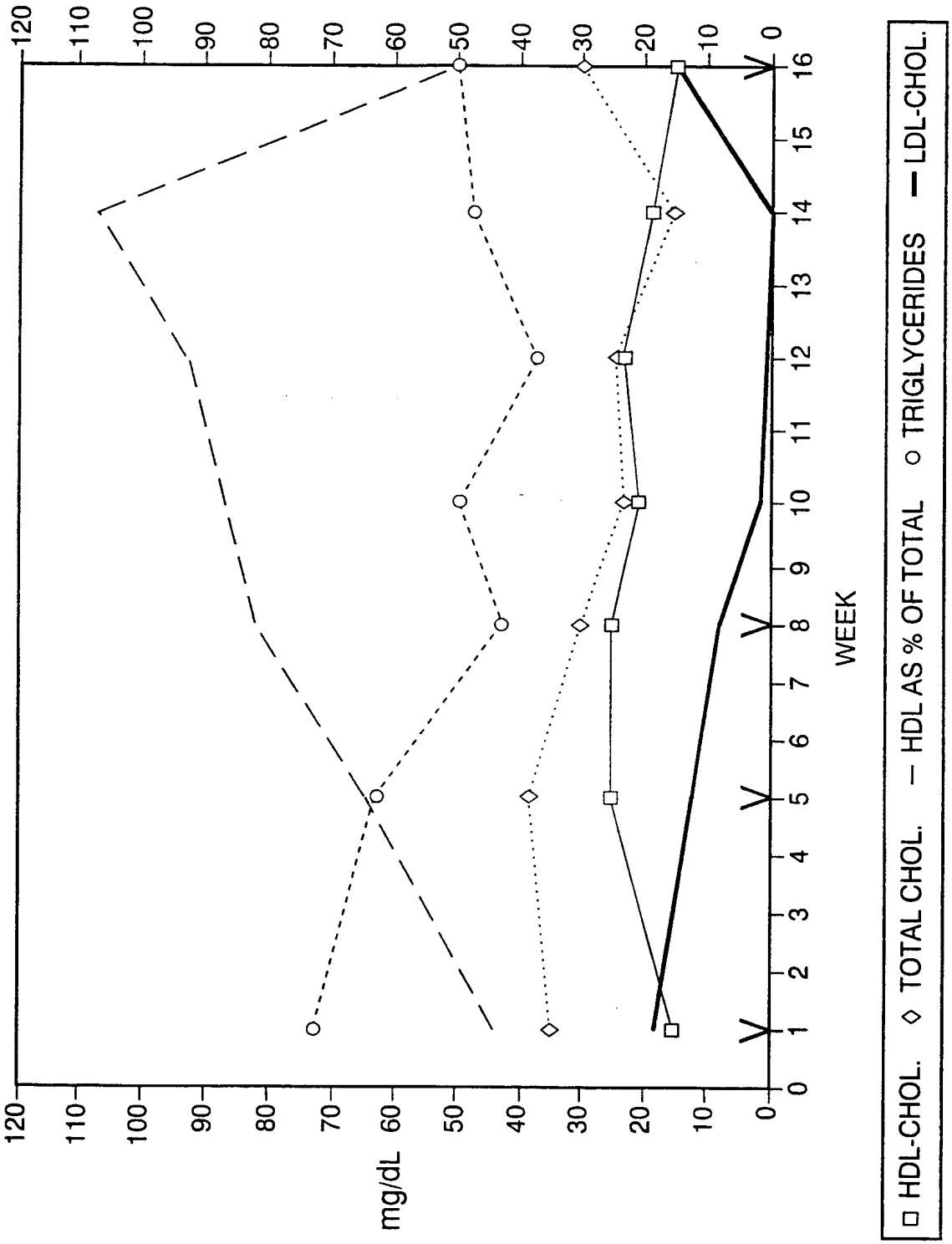

mg/dL
WEEK
□ HDL-CHOL. ◇ TOTAL CHOL. — HDL AS % OF TOTAL ○ TRIGLYCERIDES ▬ LDL-CHOL.

FIG. 8

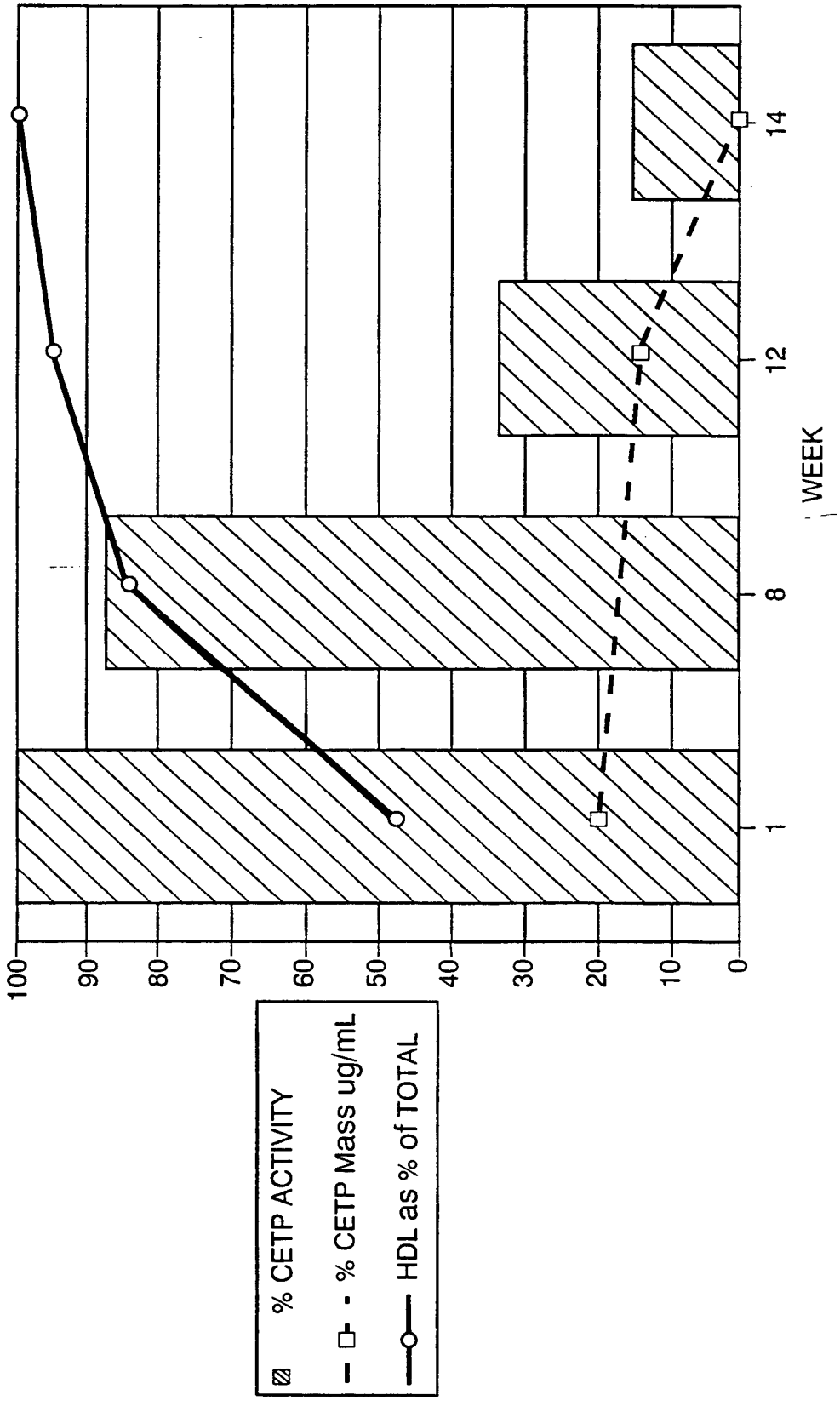

100
90
80
70
60
50
40
30
20
10
0
1
8
12
14
WEEK
% CETP ACTIVITY
% CETP Mass ug/mL
HDL as % of TOTAL

FIG. 9

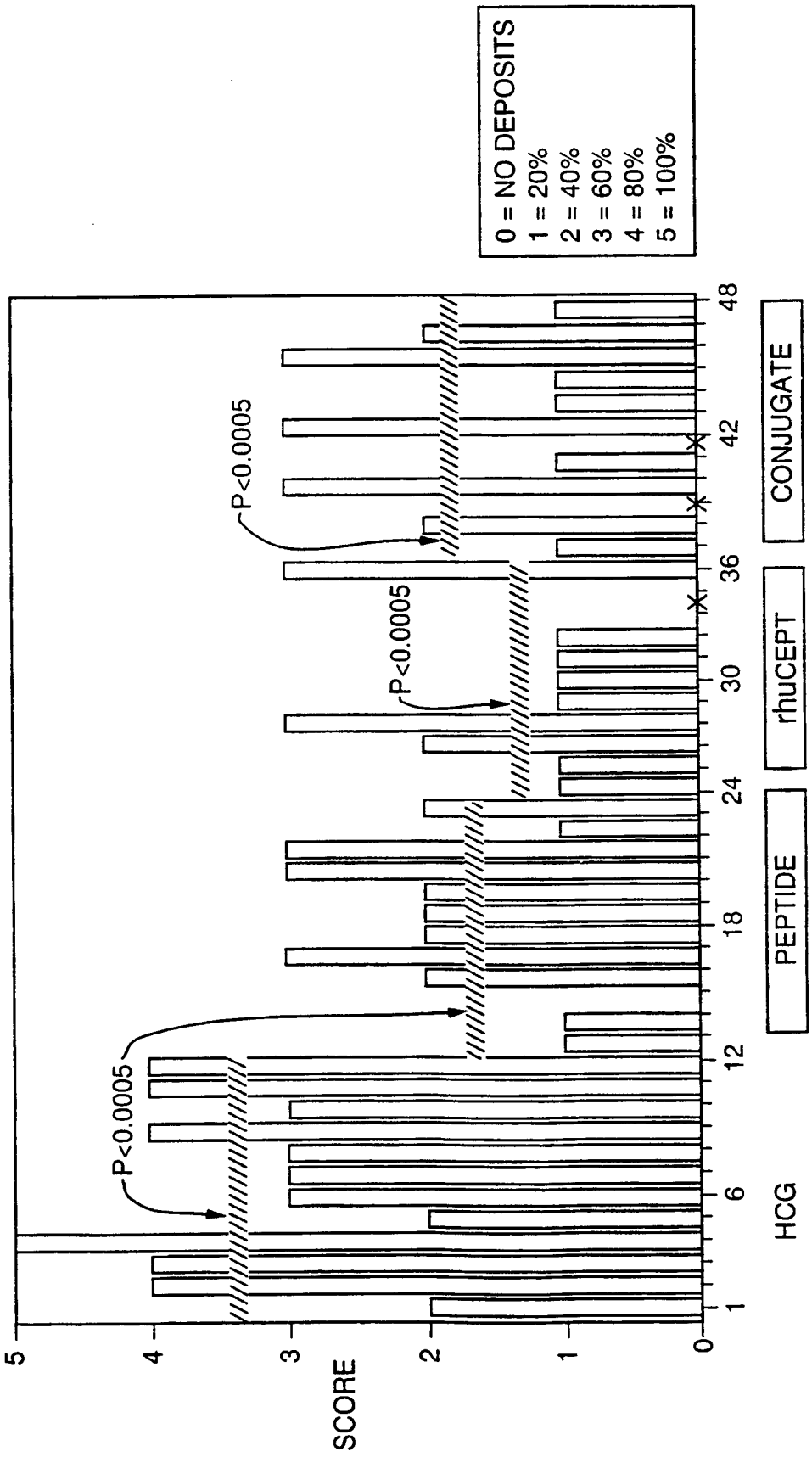

0 = NO DEPOSITS
1 = 20%
2 = 40%
3 = 60%
4 = 80%
5 = 100%
P<0.0005
P<0.0005
P<0.0005
SCORE
RABBIT #
HCG
PEPTIDE
rhuCEPT
CONJUGATE

FIG. 10

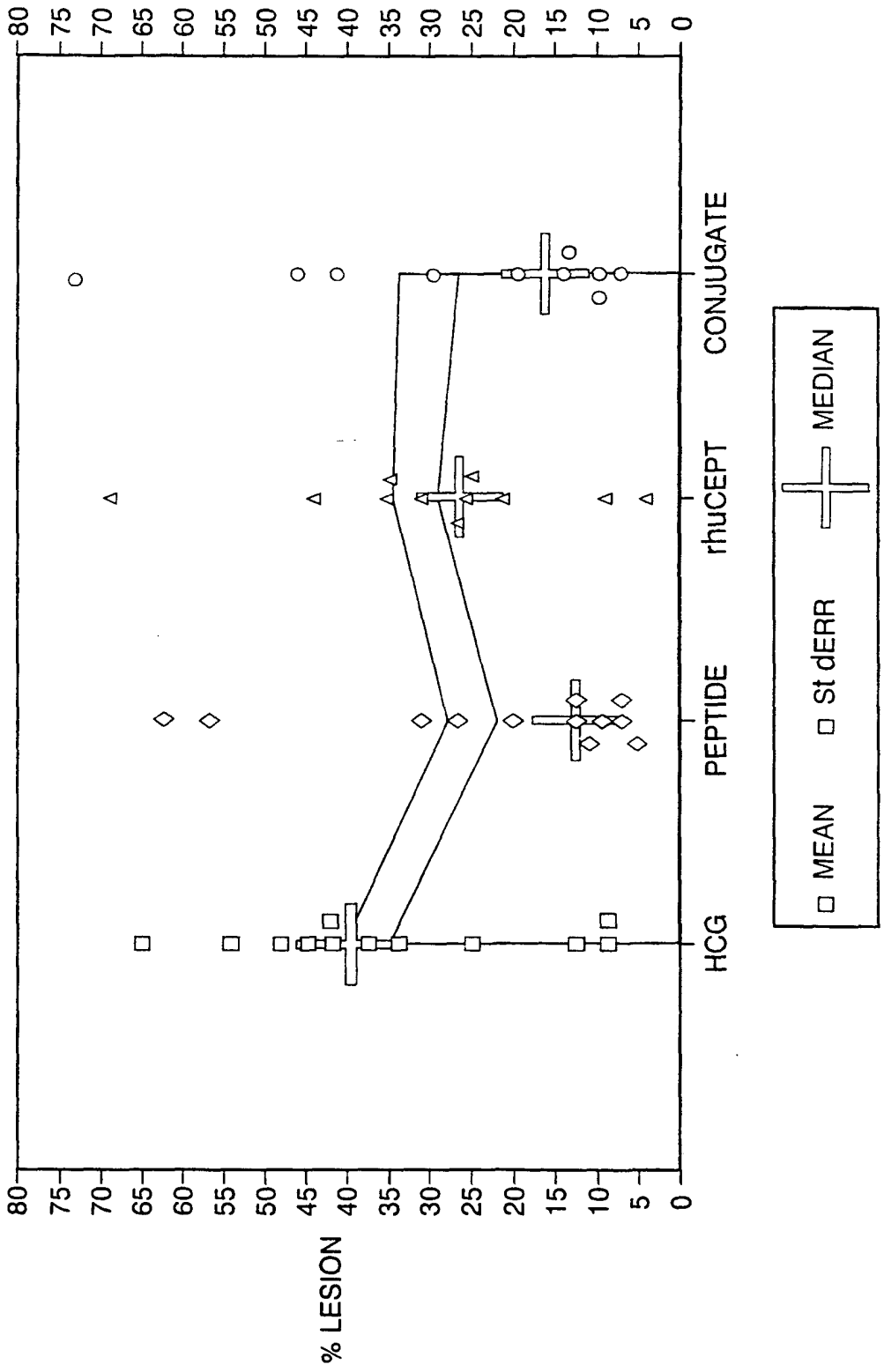

% LESION
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
HCG
PEPTIDE
rhuCEPT
CONJUGATE
MEAN
St dERR
MEDIAN

FIG. 11

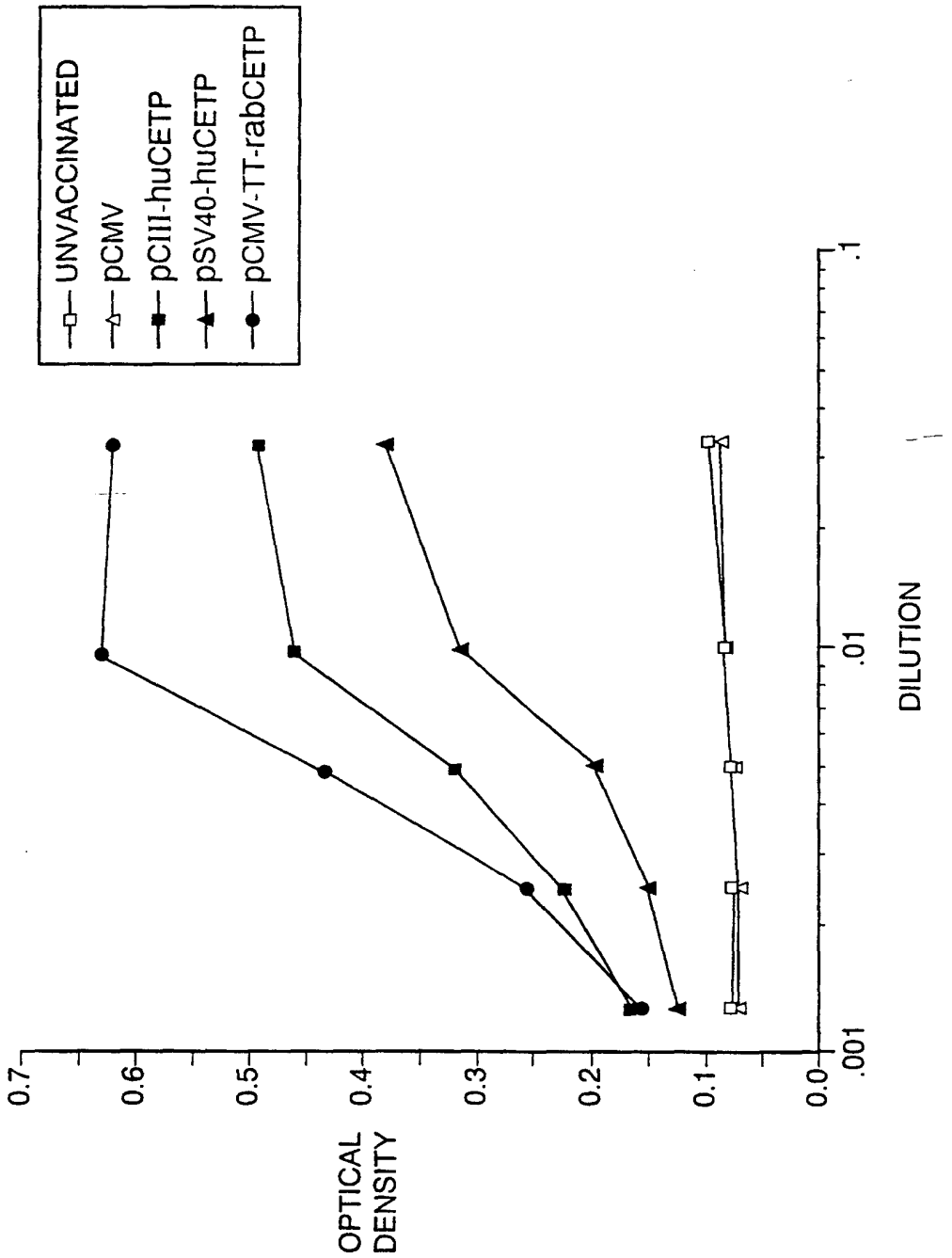

UNVACCINATED
pCMV
pCIII-huCETP
pSV40-huCETP
pCMV-TT-rabCETP
OPTICAL DENSITY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.001
.01
.1
DILUTION

FIG. 12

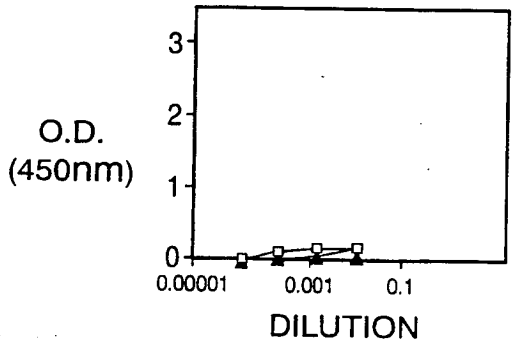


FIG. 13A

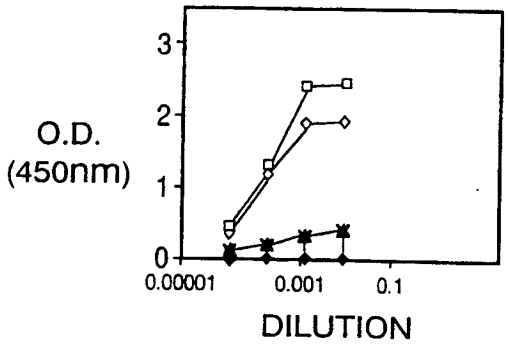


FIG. 13B

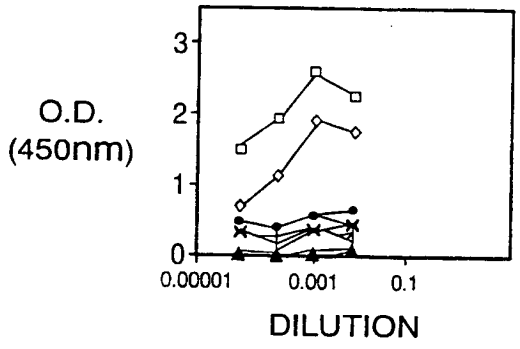


FIG. 13C

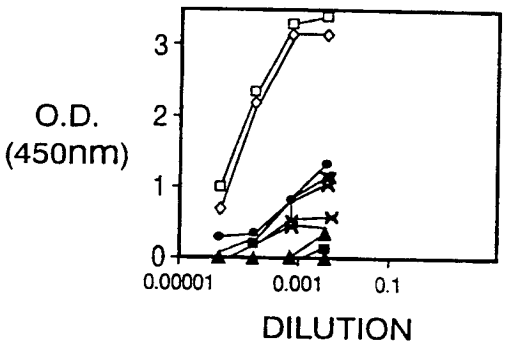


FIG. 13D

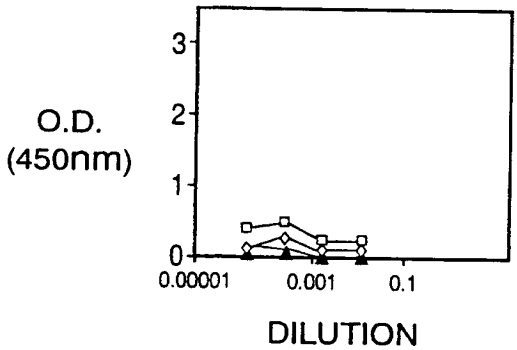


FIG. 13E

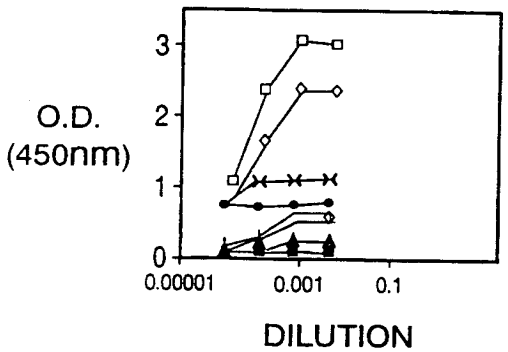
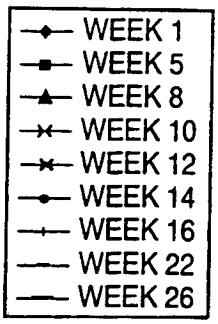


FIG. 13F



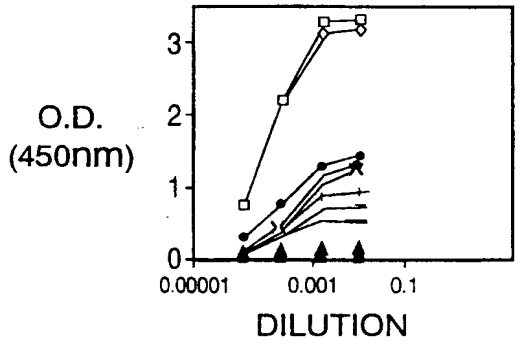


FIG. 13G

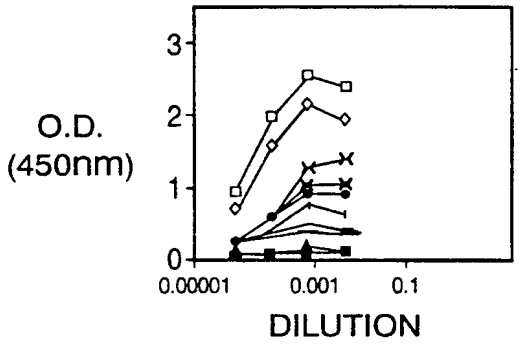


FIG. 13H

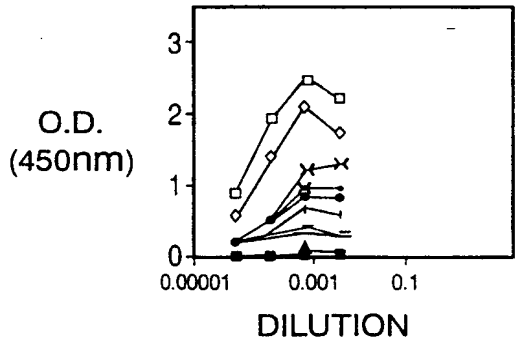


FIG. 13I

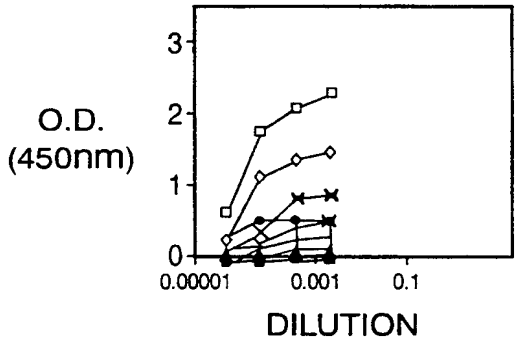


FIG. 13J

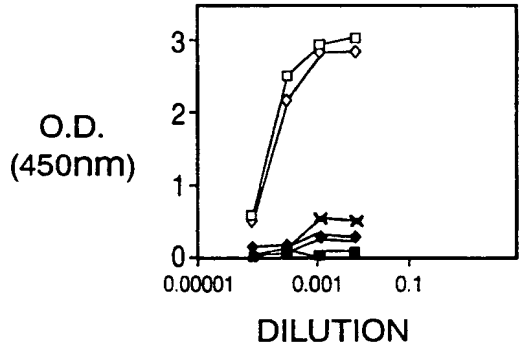
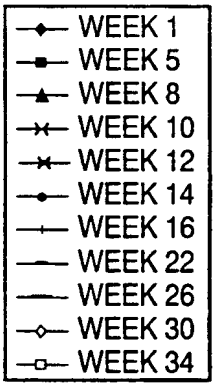


FIG. 13K



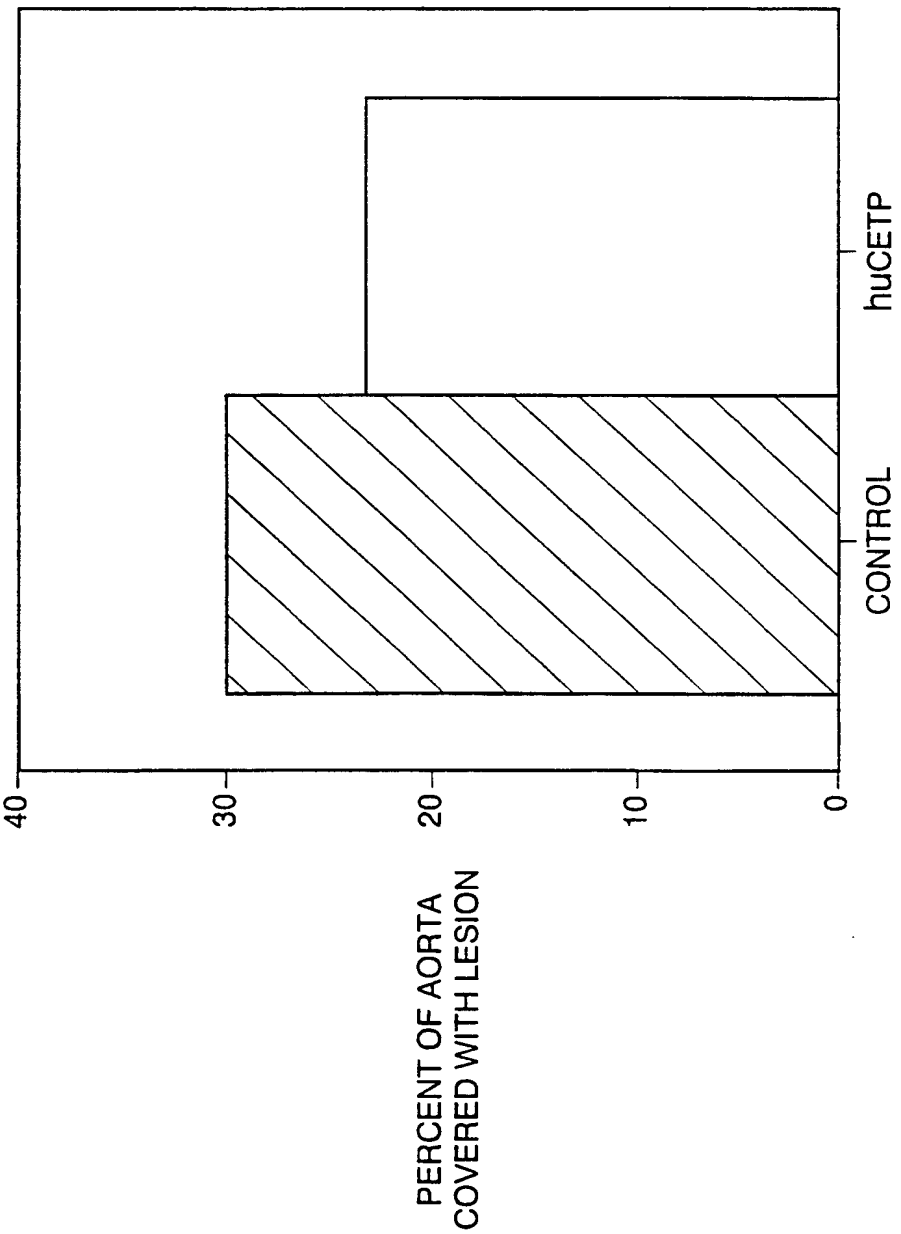

PERCENT OF AORTA
COVERED WITH LESION
40
30
20
10
0
CONTROL
huCETP

FIG. 14

SEQUENCE LISTING

<110> Rittershaus, Charles W.
Thomas, Lawrence J.
Avant Immunotherapeutics, Inc.

<120> Xenogeneic Cholesteryl Ester Transfer Protein (CETP)
for Modulation of CETP Activity

<130> TCS-420.1 PCT seqlist

<140> PCT/US98/22145
<141> 1998-10-20

<150> 08/954,643
<151> 1997-10-20

<160> 7

<170> PatentIn Ver. 2.0

<210> 1
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<302> Cloning and Sequencing of Human Cholesteryl Ester
Transfer cDNA
<303> Nature
<304> 327
<306> 632-634
<307> 1987-06-18
<313> 1 TO 476

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Cys	Ser	Lys	Gly	Thr	Ser	His	Glu	Ala	Gly	Ile	Val	Cys	Arg	Ile	Thr
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Lys	Pro	Ala	Leu	Leu	Val	Leu	Asn	His	Glu	Thr	Ala	Lys	Val	Ile	Gln
		20					25					30			

Thr	Ala	Phe	Gln	Arg	Ala	Ser	Tyr	Pro	Asp	Ile	Thr	Gly	Glu	Lys	Ala
		35					40					45			

Met	Met	Leu	Leu	Gly	Gln	Val	Lys	Tyr	Gly	Leu	His	Asn	Ile	Gln	Ile
	50					55					60				

```
Ser His Leu Ser Ile Ala Ser Ser Gln Val Glu Leu Val Glu Ala Lys
 65                  70                  75                  80

Ser Ile Asp Val Ser Ile Gln Asn Val Ser Val Val Phe Lys Gly Thr
                 85                  90                  95

Leu Lys Tyr Gly Tyr Thr Thr Ala Trp Trp Leu Gly Ile Asp Gln Ser
            100                 105                 110

Ile Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile Asn Thr Gln
        115                 120                 125

Leu Thr Cys Asp Ser Gly Arg Val Arg Thr Asp Ala Pro Asp Cys Tyr
    130                 135                 140

Leu Ser Phe His Lys Leu Leu Leu His Leu Gln Gly Glu Arg Glu Pro
145                 150                 155                 160

Gly Trp Ile Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu Lys
                165                 170                 175

Leu Val Leu Lys Gly Gln Ile Cys Lys Glu Ile Asn Val Ile Ser Asn
            180                 185                 190

Ile Met Ala Asp Phe Val Gln Thr Arg Ala Ala Ser Ile Leu Ser Asp
        195                 200                 205

Gly Asp Ile Gly Val Asp Ile Ser Leu Thr Gly Asp Pro Val Ile Thr
    210                 215                 220

Ala Ser Tyr Leu Glu Ser His His Lys Gly His Phe Ile Tyr Lys Asn
225                 230                 235                 240

Val Ser Glu Asp Leu Pro Leu Pro Thr Phe Ser Pro Thr Leu Leu Gly
                245                 250                 255

Asp Ser Arg Met Leu Tyr Phe Trp Phe Ser Glu Arg Val Phe His Ser
            260                 265                 270

Leu Ala Lys Val Ala Phe Gln Asp Gly Arg Leu Met Leu Ser Leu Met
        275                 280                 285

Gly Asp Glu Phe Lys Ala Val Leu Glu Thr Trp Gly Phe Asn Thr Asn
    290                 295                 300

Gln Glu Ile Phe Gln Glu Val Val Gly Gly Phe Pro Ser Gln Ala Gln
305                 310                 315                 320
```

```
Val Thr Val His Cys Leu Lys Met Pro Lys Ile Ser Cys Gln Asn Lys
                325                 330                 335

Gly Val Val Val Asn Ser Ser Val Met Val Lys Phe Leu Phe Pro Arg
            340                 345                 350

Pro Asp Gln Gln His Ser Val Ala Tyr Thr Phe Glu Glu Asp Ile Val
        355                 360                 365

Thr Thr Val Gln Ala Ser Tyr Ser Lys Lys Lys Leu Phe Leu Ser Leu
    370                 375                 380

Leu Asp Phe Gln Ile Thr Pro Lys Thr Val Ser Asn Leu Thr Glu Ser
385                 390                 395                 400

Ser Ser Glu Ser Ile Gln Ser Phe Leu Gln Ser Met Ile Thr Ala Val
                405                 410                 415

Gly Ile Pro Glu Val Met Ser Arg Leu Glu Val Val Phe Thr Ala Leu
            420                 425                 430

Met Asn Ser Lys Gly Val Ser Leu Phe Asp Ile Ile Asn Pro Glu Ile
        435                 440                 445

Ile Thr Arg Asp Gly Phe Leu Leu Leu Gln Met Asp Phe Gly Phe Pro
    450                 455                 460

Glu His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
465                 470                 475
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<302> Cloning and Sequencing of Human Cholesteryl Ester
Transfer cDNA
<303> Nature
<304> 327
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<307> 1987-06-18
<313> 1 TO 476

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ccagatatca cgggcgagaa ggccatgatg ctccttggcc aagtcaagta tgggttgcac 180
aacatccaga tcagccactt gtccatcgcc agcagccagg tggagctggt ggaagccaag 240
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ctgtacttct ggttctctga gcgagtcttc cactcgctgg ccaaggtagc tttccaggat 840
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gtcaccgtcc actgcctcaa gatgcccaag atctcctgcc aaaacaaggg agtcgtggtc 1020
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gtcatgtctc ggctcgaggt agtgtttaca gccctcatga acagcaaagg cgtgagcctc 1320
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<301> Nagashima, Mariko
<302> Cloning and mRNA tissue distribution of rabbit
cholesteryl ester transfer protein
<303> J. Lipid Res.
<304> 29
<306> 1643-1649
<307> 1988
<313> 1 TO 496

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 1               5                  10                  15

Lys Pro Ala Leu Leu Val Leu Asn Gln Glu Thr Ala Lys Val Val Gln
            20                  25                  30

Thr Ala Phe Gln Arg Ala Gly Tyr Pro Asp Val Ser Gly Glu Arg Ala
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```
         35                  40                  45

Val Met Leu Leu Gly Arg Val Lys Tyr Gly Leu His Asn Leu Gln Ile
     50                  55                  60

Ser His Leu Ser Ile Ala Ser Ser Gln Val Glu Leu Val Asp Ala Lys
 65                  70                  75                  80

Thr Ile Asp Val Ala Ile Gln Asn Val Ser Val Val Phe Lys Gly Thr
                 85                  90                  95

Leu Asn Tyr Ser Tyr Thr Ser Ala Trp Gly Leu Gly Ile Asn Gln Ser
            100                 105                 110

Val Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile Asn Thr Glu
        115                 120                 125

Leu Thr Cys Asp Ala Gly Ser Val Arg Thr Asn Ala Pro Asp Cys Tyr
    130                 135                 140

Leu Ala Phe His Lys Leu Leu Leu His Leu Gln Gly Glu Arg Glu Pro
145                 150                 155                 160

Gly Trp Leu Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu Lys
                165                 170                 175

Leu Ile Leu Lys Arg Gln Val Cys Asn Glu Ile Asn Thr Ile Ser Asn
            180                 185                 190

Ile Met Ala Asp Phe Val Gln Thr Arg Ala Ala Ser Ile Leu Ser Asp
        195                 200                 205

Gly Asp Ile Gly Val Asp Ile Ser Val Thr Gly Ala Pro Val Ile Thr
    210                 215                 220

Ala Thr Tyr Leu Glu Ser His His Lys Gly His Phe Thr His Lys Asn
225                 230                 235                 240

Val Ser Glu Ala Phe Pro Leu Arg Ala Phe Pro Pro Gly Leu Leu Gly
                245                 250                 255

Asp Ser Arg Met Leu Tyr Phe Trp Phe Ser Asp Gln Val Leu Asn Ser
            260                 265                 270

Leu Ala Arg Ala Ala Phe Gln Glu Gly Arg Leu Val Leu Ser Leu Thr
        275                 280                 285

Gly Asp Glu Phe Lys Lys Val Leu Glu Thr Gln Gly Phe Asp Thr Asn
```

290 295 300

Gln Glu Ile Phe Gln Glu Leu Ser Arg Gly Leu Pro Thr Gly Gln Ala
305 310 315 320

Gln Val Ala Val His Cys Leu Lys Val Pro Lys Ile Ser Cys Gln Asn
325 330 335

Arg Gly Val Val Val Ser Ser Ser Val Ala Val Thr Phe Arg Phe Pro
340 345 350

Arg Pro Asp Gly Arg Glu Ala Val Ala Tyr Arg Phe Glu Glu Asp Ile
355 360 365

Ile Thr Thr Val Gln Ala Ser Tyr Ser Gln Lys Lys Leu Phe Leu His
370 375 380

Leu Leu Asp Phe Gln Cys Val Pro Ala Ser Gly Arg Ala Gly Ser Ser
385 390 395 400

Ala Asn Leu Ser Val Ala Leu Arg Thr Glu Ala Lys Ala Val Ser Asn
405 410 415

Leu Thr Glu Ser Arg Ser Glu Ser Leu Gln Ser Ser Leu Arg Ser Leu
420 425 430

Ile Ala Thr Val Gly Ile Pro Glu Val Met Ser Arg Leu Glu Val Ala
435 440 445

Phe Thr Ala Leu Met Asn Ser Lys Gly Leu Asp Leu Phe Glu Ile Ile
450 455 460

Asn Pro Glu Ile Ile Thr Leu Asp Gly Cys Leu Leu Leu Gln Met Asp
465 470 475 480

Phe Gly Phe Pro Lys His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
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<301> Nagashima, Mariko
<302> Cloning and mRNA tissue distribution of rabbit
cholesteryl ester transfer protein
<303> J. Lipid Res.
<304> 29
<306> 1643-1649
<307> 1988
<313> 1 TO 496

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agggccgcca gcatcctctc agatggagac atcggggtgg acatttccgt gacgggggcc 660
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<210> 5
<211> 477
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: humanized
rabbit CETP protein

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Lys Pro Ala Leu Leu Val Leu Asn Gln Glu Thr Ala Lys Val Val Gln
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Thr Ala Phe Gln Arg Ala Gly Tyr Pro Asp Val Ser Gly Glu Arg Ala
35 40 45

Val Met Leu Leu Gly Arg Val Lys Tyr Gly Leu His Asn Leu Gln Ile
50 55 60

Ser His Leu Ser Ile Ala Ser Ser Gln Val Glu Leu Val Asp Ala Lys
65 70 75 80

Thr Ile Asp Val Ala Ile Gln Asn Val Ser Val Val Phe Lys Gly Thr
85 90 95

Leu Asn Tyr Ser Tyr Thr Ser Ala Trp Gly Leu Gly Ile Asn Gln Ser
100 105 110

Val Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile Asn Thr Glu
115 120 125

Leu Thr Cys Asp Ala Gly Ser Val Arg Thr Asn Ala Pro Asp Cys Tyr
130 135 140

Leu Ala Phe His Lys Leu Leu Leu His Leu Gln Gly Glu Arg Glu Pro
145 150 155 160

Gly Trp Leu Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu Lys
165 170 175

Leu Ile Leu Lys Arg Gln Val Cys Asn Glu Ile Asn Thr Ile Ser Asn
180 185 190

Ile Met Ala Asp Phe Val Gln Thr Arg Ala Ala Ser Ile Leu Ser Asp
195 200 205

Gly Asp Ile Gly Val Asp Ile Ser Val Thr Gly Ala Pro Val Ile Thr
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Ala Thr Tyr Leu Glu Ser His His Lys Gly His Phe Thr His Lys Asn
225 230 235 240

Val Ser Glu Ala Phe Pro Leu Arg Ala Phe Pro Pro Gly Leu Leu Gly
245 250 255

Asp Ser Arg Met Leu Tyr Phe Trp Phe Ser Asp Gln Val Leu Asn Ser
260 265 270

Leu Ala Arg Ala Ala Phe Gln Glu Gly Arg Leu Val Leu Ser Leu Thr
275 280 285

Gly Asp Glu Phe Lys Lys Val Leu Glu Thr Gln Gly Phe Asp Thr Asn
290 295 300

Gln Glu Ile Phe Gln Glu Leu Ser Arg Gly Leu Pro Thr Gly Gln Ala
305 310 315 320

Gln Val Ala Val His Cys Leu Lys Val Pro Lys Ile Ser Cys Gln Asn
325 330 335

Arg Gly Val Val Val Ser Ser Ser Val Ala Val Thr Phe Arg Phe Pro
340 345 350

Arg Pro Asp Gly Arg Glu Ala Val Ala Tyr Arg Phe Glu Glu Asp Ile
355 360 365

Ile Thr Thr Val Gln Ala Ser Tyr Ser Gln Lys Lys Leu Phe Leu His
370 375 380

Leu Leu Asp Phe Gln Cys Val Pro Lys Ala Val Ser Asn Leu Thr Glu
385 390 395 400

Ser Arg Ser Glu Ser Leu Gln Ser Ser Leu Arg Ser Leu Ile Ala Thr
405 410 415

Val Gly Ile Pro Glu Val Met Ser Arg Leu Glu Val Ala Phe Thr Ala
420 425 430

Leu Met Asn Ser Lys Gly Leu Asp Leu Phe Glu Ile Ile Asn Pro Glu
435 440 445

Ile Ile Thr Leu Asp Gly Cys Leu Leu Leu Gln Met Asp Phe Gly Phe
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Pro Lys His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
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<210> 6
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: humanized

rabbit CETP protein

<400> 6

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Lys Pro Ala Leu Leu Val Leu Asn Gln Glu Thr Ala Lys Val Val Gln
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Thr Ala Phe Gln Arg Ala Gly Tyr Pro Asp Val Ser Gly Glu Arg Ala
        35                  40                  45

Val Met Leu Leu Gly Arg Val Lys Tyr Gly Leu His Asn Leu Gln Ile
    50                  55                  60

Ser His Leu Ser Ile Ala Ser Ser Gln Val Glu Leu Val Asp Ala Lys
65                  70                  75                  80

Thr Ile Asp Val Ala Ile Gln Asn Val Ser Val Val Phe Lys Gly Thr
                85                  90                  95

Leu Asn Tyr Ser Tyr Thr Ser Ala Trp Gly Leu Gly Ile Asn Gln Ser
            100                 105                 110

Val Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile Asn Thr Glu
        115                 120                 125

Leu Thr Cys Asp Ala Gly Ser Val Arg Thr Asn Ala Pro Asp Cys Tyr
    130                 135                 140

Leu Ala Phe His Lys Leu Leu Leu His Leu Gln Gly Glu Arg Glu Pro
145                 150                 155                 160

Gly Trp Leu Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu Lys
                165                 170                 175

Leu Ile Leu Lys Arg Gln Val Cys Asn Glu Ile Asn Thr Ile Ser Asn
            180                 185                 190

Ile Met Ala Asp Phe Val Gln Thr Arg Ala Ala Ser Ile Leu Ser Asp
        195                 200                 205

Gly Asp Ile Gly Val Asp Ile Ser Val Thr Gly Ala Pro Val Ile Thr
    210                 215                 220

Ala Thr Tyr Leu Glu Ser His His Lys Gly His Phe Thr His Lys Asn
225                 230                 235                 240
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Val Ser Glu Ala Phe Pro Leu Arg Ala Phe Pro Pro Gly Leu Leu Gly
245 250 255

Asp Ser Arg Met Leu Tyr Phe Trp Phe Ser Asp Gln Val Leu Asn Ser
260 265 270

Leu Ala Arg Ala Ala Phe Gln Glu Gly Arg Leu Val Leu Ser Leu Thr
275 280 285

Gly Asp Glu Phe Lys Lys Val Leu Glu Thr Gln Gly Phe Asp Thr Asn
290 295 300

Gln Glu Ile Phe Gln Glu Leu Ser Arg Gly Leu Pro Thr Gly Gln Ala
305 310 315 320

Gln Val Ala Val His Cys Leu Lys Val Pro Lys Ile Ser Cys Gln Asn
325 330 335

Arg Gly Val Val Val Ser Ser Ser Val Ala Val Thr Phe Arg Phe Pro
340 345 350

Arg Pro Asp Gly Arg Glu Ala Val Ala Tyr Arg Phe Glu Glu Asp Ile
355 360 365

Ile Thr Thr Val Gln Ala Ser Tyr Ser Gln Lys Lys Leu Phe Leu His
370 375 380

Leu Leu Asp Phe Gln Cys Val Pro Ala Ser Gly Arg Ala Gly Ser Ser
385 390 395 400

Ala Asn Leu Ser Val Ala Leu Arg Thr Glu Ala Lys Ala Val Ser Asn
405 410 415

Leu Thr Glu Ser Arg Ser Glu Ser Leu Gln Ser Ser Leu Arg Ser Leu
420 425 430

Ile Ala Thr Val Gly Ile Pro Glu Val Met Ser Arg Leu Glu Val Ala
435 440 445

Phe Thr Ala Leu Met Asn Ser Lys Gly Leu Asp Leu Phe Glu Ile Ile
450 455 460

Asn Pro Glu Ile Ile Thr Leu Asp Gly Cys Leu Leu Leu Gln Met Asp
465 470 475 480

Phe Gly Phe Pro Glu His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
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<210> 7
<211> 31
<212> PRT
<213> Artificial Sequence

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<223> Description of Artificial Sequence: fusion protein
of a tetanus toxoid segment and human CETP
C-terminus

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1 5 10 15

Gly Phe Pro Glu His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
20 25 30

INTERNATIONAL SEARCH REPORT

Interna[redacted]pplication No
PCT/U[redacted]8/22145

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 A61K39/39 A61K48/00 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HESLER C. ET AL.,: "Purification and characterization of a human plasma cholesteryl ester transfer protein" J. BIOL. CHEM, vol. 262, no. 5, - 15 February 1987 pages 2275-2282, XP002096997 see whole doc., esp. P.2282 2.col. ---	1,3,6,15
X	SMITH A M ET AL: "PREPARATION OF AN ANTI-PEPTIDE ANTISERUM SPECIFIC FOR CHOLESTERYL ESTER TRANSFER PROTEIN (CEPT)" MEDICAL SCIENCE RESEARCH, vol. 21, no. 24, 16 December 1993, page 911/912 XP002041707 see the whole document --- -/--	1,3,7,15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 1999	01/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat[redacted] Application No
PCT/[redacted]98/22145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39168 A (IMMUNE RESPONSE CORP INC) 12 December 1996 SEE WHOLE DOC., esp. page 1,2 and claims	1-3,7, 15-21
X	WO 96 34888 A (T CELL SCIENCES INC ;RITTERSHAUS CHARLES W (US); THOMAS LAWRENCE J) 7 November 1996 cited in the application see whole doc. esp. claims and examples	1-3,6,7, 15-17, 20,21
A	HESLER C. ET AL.,: "Monoclonal antibodies to the Mr 74000 cholesteryl ester transfer protein neutralize all of the cholesteryl ester and triglyceride transfer activities in human plasma" J. BIOL. CHEM., vol. 263, no. 11, - 15 April 1988 pages 5020-5023, XP002096998	1-37
P,X	WO 97 41227 A (T CELL SCIENCES INC ;THOMAS LAWRENCE J (US)) 6 November 1997 cited in the application see the whole document	8-14, 26-37
P,X	THOMAS L.J. ET AL.,: "Use of xenogeneic cholesteryl ester transfer protein (CETP) in a plasmid-based vaccine to produce anti-cetp autoantibodies for the prevention/treatment of atherosclerosis" FASEB JOURNAL, vol. 12, no. 4, - 17 March 1998 page a310,1805 XP002096999 see the whole document	8-14, 26-37

1

INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/US 98/ 22145

Box I Observations where certain claims wer found uns archable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 15-37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/22145

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639168 A	12-12-1996	AU 6091296 A	24-12-1996
		CA 2223177 A	12-12-1996
		EP 0831881 A	01-04-1998
WO 9634888 A	07-11-1996	AU 5636096 A	21-11-1996
		CA 2219795 A	07-11-1996
		EP 0827509 A	11-03-1998
WO 9741227 A	06-11-1997	AU 2994697 A	19-11-1997